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Work performed in this grant continues to address major hurdles in the development of an effective synthetic peptide HIV vaccine: 1) the ability of synthetic peptides to mimic conformational epitopes within the HIV envelope protein and induce antibody responses capable of neutralizing primary HIV isolates and 2) the design of synthetic peptide immunogens capable of being recognized by MHC Class I and II molecules in outbred populations. In technical aim #1, computer analysis of the envelope protein sequence of CCR5-utilizing and CXCR4-utlizing HIV isolates has allowed us to synthesize novel HIV synthetic peptides that contain consensus sequences specific for CCR5-utililizing and CXCR4-utilizing viruses. One new peptide identified in aim #1, C4E9V-SP788-89.6P, has induced sera capable of neutralizing SHIV-89.6 and the pathogenic SHIV-KB9 cloned virus. In technical aim #2, C4E9V-SP788-89.6P has been synthesized and prepared for structural studies. In technical aim #3, MHC Class I binding epitopes within the C4-V3 peptides have been identified for a variety of different HLA molecules.

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DAMD17-94-J-4467 PROGRESS REPORT - YEAR 4: STRUCTURAL AND FUNCTIONAL STUDIES OF EXPERIMENTAL HIV SYNTHETIC PEPTIDE IMMUNOGENS

Technical Aim #1. Use existing and new human and mouse monoclonal antibodies (mAbs) as templates for definition of native gp120 conformations and determinants of synthetic peptides that bind to these mAbs.

Investigators: Barton F. Haynes, M.D., Larry Liao, M.D., Ph.D., and Herman F. Staats, Ph.D.

Introduction: The hypotheses to be tested in Technical Aim 1 are:

1. Regarding the B cell anti-HIV immune responses, synthetic peptides can be designed that mimic not only linear determinants of neutralizing epitopes of gp120 but also mimic more conserved, broadly neutralizing HIV conformational determinants.

2. Regarding T cell anti-HIV responses, synthetic peptides can be designed that optimize antigen processing and/or antigen-presentation by MHC molecules on antigen-presenting cells.

3. Regarding MHC-restricted binding of HIV peptides, the HIV synthetic peptide mixtures can be designed to stimulate T cell responses in individuals of ethically diverse ancestry in multiple geographic locations.

Body

Identification of possible regions in HIV-1 envelope protein involved in the interaction between HIV envelope protein and chemokine receptor.

Chemokine receptors CCR5 and CXCR4 have been demonstrated as the major co-receptors for HIV-1. This has provided a new framework for understanding viral tropism and pathogenesis at the molecular level. It has been shown that the HIV-1 envelope protein gp120 V3 region plays a critical role in determination of co-receptor usage. In this regard, monocyte tropic HIV isolates and most of HIV-1 primary isolates use CCR5 as a co-receptor. Studies have demonstrated that the envelope of HIV primary isolates differs from the envelope of T cell-line adapted (TCLA) HIV strains, and that antibodies that neutralize TCLA HIV viruses poorly neutralize moncytotropic or primary isolates.

In order to target the region(s) of HIV-1 envelope that interact with chemokine receptors, and develop a better strategy to neutralize HIV-1 primary isolates, we have conducted sequence analysis of HIV isolates that use either CCR5 or CXCR4 as co-receptors. To generate the consensus sequence between HIV isolates that use either CCR5 or CXCR4 as co-receptor, we have downloaded the envelope protein sequences of HIV isolates that have been studied for their

chemokine co-receptor usage and described by Doms and Moore through the HIV sequence database (1). We have compiled the HIV envelope protein sequences from 17 HIV isolates that use CCR5 as co-receptor, and 6 HIV isolates that use CXCR4 as co-receptor (Table 1). The consensus sequences of the envelope protein from these two groups of HIV isolates were generated using computer software in the Wisconsin Package Version 9.1, Genetic Computer Group (GCG), Madison, Wisc. Similarly, the consensus sequences of HIV envelope proteins from the CCR5-utilizing isolates and CXCR4-utilizing isolates were compared by using GCG computer software. Consistent variations can be identified in the V2 and V3 loop regions between these two groups (Table 2). A similar finding of these consensus variations has been reported by study of in vivo evolution of HIV-1 co-receptor usage (2). In addition, differences in the N-terminal region between these groups of HIV isolates have been noted (Table 2). Although variations in amino acid sequence have been also observed in other regions of the HIV envelope protein, no consistent similarities among the group of isolates using either CCR5 or CXCR4 receptor were identified.

Design of synthetic peptides reflecting regions of HIV gp120 that are involved on gp120-chemokine co-receptor interactions. Based on the consensus sequence analysis of the envelope protein from CCR5-utlizing and CXCR4-utlizing HIV isolates, we have designed a new panel of peptide immunogens to expand our efforts to neutralize HIV-1 primary isolates. Table 3 lists the peptide sequences and designs. Figure 1 shows the locations of these peptides in HIV gp120. We have begun to study the immunogenicity of these peptide in guinea pigs, and evaluate the ability of antisera raised against the individual peptides or combination of the new V2 and V3 peptides to neutralize HIV primary isolates. As shown in Table 4, these new panel peptides are highly immunogenic in guinea pigs and induced high ELISA titers against the immunizing peptides. Antiserum from one of two guinea pigs raised against peptide C4E9V-SP780-89.6P, that was derived from the V3 region with the extended right side of the loop (see Figure 1 for the relative location), developed neutralizing antibody against HIV primary isolates 89.6. Furthermore, antiserum from guinea pig # 76 immunized with peptide C4E9V-SP788-89.6P, derived from the entire V3 loop region (see Figure 1 for the relative location) not only developed neutralizing antibody against SHIV 89.6 but also neutralized the pathogenic strain of SHIV 89.6P. Studies to evaluate these new peptides for their ability to induce neutralization antibodies against HIV primary are still in progress.

Intranasal immunization with HIV synthetic peptides

We have continued with theme of mucosal immunization using synthetic peptide immunogens. Our hypothesis is that the formulation of the peptide may affect its ability to induce anti-HIV neutralizing antibody responses. For example, subcutaneous immunization with the peptide formulated in mineral oil may induce qualitatively different antibody responses than those induced by intranasal immunization with the peptide formulated in water. Therefore, the synthetic peptide immunogen C4E9V-SP788-89.6P was tested for its ability to induce serum anti-peptide and anti-HIV neutralizing antibody responses after intranasal immunization of rabbits. Rabbits were intranasally immunized with C4E9V-SP788-89.6P using a variety of different mucosal adjuvants including IL-1α, IL-1β, cholera toxin, and IL-15. Intranasal immunization with 4 doses of C4E9V-SP788-89.6P (220 μg peptide per rabbit per dose) induced high titered anti-peptide serum IgG responses (Table 5). Although high titered serum IgG responses were induced,

neutralization of SHIV-89.6 or SHIV-89.6P was not observed (Table 5). Although the rabbit anti- C4E9V-SP788-89.6P antisera induced by intranasal immunization was not able to neutralize SHIV 89.6 or 89.6P, serum from guinea pig #77 that was immunized with the C4E9V-SP788-89.6P did neutralize both SHIV-89.6 and cloned SHIV-89.6P KB9. Rabbits will continue to be intranasally immunized in an attempt to increase the ELISA titer and hopefully induce neutralizing anti-SHIV89.6 and anti-SHIV89.6P antibodies.

Conclusion

Synthetic peptides may be designed to induce anti-HIV antibodies that neutralize HIV-1 primary isolates. Additional studies must be performed to determine if synthetic peptides that correspond to different HIV-1 primary isolate V3 loops are able to induce antibodies that neutralize the corresponding virus.

Studies for the extended funding period

Because we have been able to observe neutralization of SHIV-89.6 and SHIV-KB9 after immunization with the HIV peptide C4E9V-SP788-89.6P, we will continue to evaluate the sera from animals immunized with the C4E9V-SP788-89.6P peptide alone or in combination with the V2 HIV peptides for its ability to neutralize SHIV-89.6 and/or SHIV-KB9. Peptides will also be utilized for intranasal immunization to determine if the route of immunization and the formulation of the peptide affects its ability to induce neutralizing anti-SHIV antibody responses. The ability of the peptides to induce neutralizing antibodies will be evaluated in conjunction with the structural data for C4E9V-SP788-89.6P obtained from Aim 2.

References

1. Robert W. Doms and John P. Moore. HIV-1 Coreceptor Use: A molecular window into viral tropism. HIV Sequence Database 1997, Human Retroviruses and AIDS 1997. Los Alamos National Laboratory.

2. Scarlatti, G.,Tresoldi, E., Bjorndal, A.,Fredriksson, R., Colognesi, C., Deng, H. K., Malnati, M. S., Plebani, A.,Siccardi, A. G., Littman, D. R., Fenyo, E. M. Lusso, P. In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. Nature Medicine, 1997. 3(11): p. 1259-65.

Table 1

HIV-1 Isolate Sequences Used In Analysis

Name of HIV isolate	Accession No.	Coreceptor usage
92BR025.9	U52953	CCR5
93MW965.2	U08455	CCR5
DJ259	L22940	CCR5
92TH22	U09131	CCR5
93TH966.8	U08456	CCR5
93TH976.17	08458	CCR5
92RW20	U08794	CCR5
92UG31	L34667	CCR5
92UG037	U51190	CCR5
92UG975.10	U27426	CCR5
93BR029.2	U27417	CCR5
BZ162	L22084	CCR5
92TH014.12	UO8801	CCR5
JR-CSF	M38429	CCR5
92Br20	U08997	CCR5
92US657	U04908	CCR5
91US005.11	U27434	CCR5
HXBc2	K03455	CXCR4
hivlai	X01762	CXCR4
NL4-3	M19921	CXCR4
92TH599.24	U08447	CXCR4
92UG021.16	U27399	CXCR4
ZAM20	L22956	CXCR4

Table 2

Consistent Variations in Envelope Protein Between CCR5-Utilizing and CXCR4-Utilizing HIV Isolates

HIV isolate	A.A. Sequence
HIV V2 region	
HIV/CCR5-Con1	SFNMTTELRDKKQKVYALFYKLDVVPINNNDNNSSNNSSEYRLI[1]
HIVCCR5-Con2	SFNMTTELRDKKQKVYALFYKLDVVPINNNDNNS-------YRLI[2]
HIVCCR5-Con3	SFNMTTELRDKKQKVYALFYKLDVVPIDNDNTS--------YRLI[3]
HIV/CXCR4-Con1	SFNISTSIRGKVQKEYALFYKLDIVPIDNDTTS--------YKLI[4]
HIV/CXCR4-Con2	SFNISTSIRGKVQKEYALFYKLDIVPIDNDTTSskSsr---YKLI[5]
HIV V3 region	
HIV/CCR5-Cons	KQIINMWQVVGRAMYANTRKSIRI--GPGQAFYATGDIIGDIRQAH[6]
HIV/CXCR4/Con1	KQIINMWQVVGRAMYANTRKSIRI--GPGRAFVTTG KIGDIRQAH[7]
HIVCXCR4/Cons2	KQIINMWQVVGRAMYANTRKSIRIQRGPGRAFVTTG KIGDIRQAH[8]
HIV N-terminal region	
HIV/CCR5-Cons	AEDNLWVTVYYTG[9]
HIV/CXCR4-Cons	ATEKLWVTVYYTG[10]

1.Consensus sequence in the V2 region from 17 HIV isolates using CCR5 as coreceptor. In this consensus sequence, "SSNNSSE" are present only in some of the isolates.
2. 10 out of 17 HIV isolates using CCR5 receptor do not have "SSNNSSE" sequence.
3. 4 out of 17 HIV isolates using CCR5 coreceptor have this consensus sequence which bears the same length as HIV isolates using CXCR4 coreceptor.
4. Consensus sequence in the V2 region from 6 HIV isolates using CXCR4 coreceptor. These 6 HIV isolates including 3 subclones of IIIB, HXBc2, HIVLai and NL4-3.
5. "SKSSR" sequence is present in 3 out of 6 HIV isolates using CXCR4 coreceptor, and amino acids in small letter are not consensus among those 3 isolates.
6. Consensus sequence in the V3 region from 17 HIV isolates using CCR5 coreceptor.
7. Consensus sequence in the V3 region from 6 HIV isolates using CXCR4 coreceptor.
8. Three isolates (IIIB subclones) have "QR", and 1 isolate has "GH" additional a.a in this region.
9. Consensus sequence in the N-terminal region from 17 HIV isolates using CCR5 coreceptor.
10. Consensus sequence in the N-terminal region from 6 HIV isolates using CXCR4 coreceptor.

Table 3

Amino Acid Sequence of New Panel of HIV-1 Envelope Synthetic Peptides Under Study

Name of peptide	Amino acid sequence
HIV V2 region	
S770-BAL	SFNITTNIRGKVQKEYALFYKLDIAPIDNNSNNRYRLI
SP770-89.6	SFYITTSIRNKVKKEYALFNRLDVVPIENTNNTKYRLI
SP770-89.6P	SFYITTSIRNKVKKEYALFNRLDVVPVKNTNNTKYRLI
SP770-R5-Con2	SFNMTTELRDKKQKVYALFYKLDVVPINNNDNNSYRLI
SP770-X4-Cons	SFNISTSIRGKVQKEYALFYKLDIVPIDNDTTSYKLI
HIV V3 region	
C4E9V-SP780-BAL	KQIINMWQVVGRAMYANTRKSIHIGPGRAFYTTGEIIGDIRQAH
C4E9V-SP780-89.6	KQIINMWQVVGRAMYANTRRRLSIGPGRAFYARRNIIGDIRQAH
C4E9V-SP780-89.6P	KQIINMWQVVGRAMYANTRERLSIGPGRAFYARRNIIGDIRQAH
SP780-R5/Cons	KQIINMWQVVGRAMYANTRKSIRIGPGQAFYATGDIIGDIRQAH
SP780-X4/Cons	KQIINMWQVVGRAMYANTRKSIRIQRGPGRAFVTTGKIGDIRQAH
C4E9V-SP788-89.6P	KQIINMWQVVGRAMYATRPNNNTRERLSIGPGRAFYARRNIIGDIRQA
HIV N-terminal region	
SP500-R5/Cons	KQIINMWQVVGRAMYAAEDNLWVTVYYTG
SP500-X4/Cons	KQIINMWQVVGRAMYAATEKLWVTVYYTG

C4 Th gp120 sequences when present, are underlined.

Table 4

ELISA and neutralization assay summary of guinea pig antisera raised against SHIV 89.6 envelope

G. Pig No.	Immunogen	Bleed No.	ELISA Titer	Neutralization in MT-2 (SHIV89.6)	Neutralization in MT-2 (SHIV-KB9)
75	C4E9V-SP788-89.6P	3	409600	<30	<30
76	C4E9V-SP788-89.6P	3	819200	175	5
77	C4E9V-SP780-BAL	3	n/d	<30	<30
78	C4E9V-SP780-BAL	3	n/d	<30*	<30*
79	SP770-BAL	3	51200	<30*	<30*
80	SP770-BAL	3	409600	<30	<30
81	C4E9V-SP780-89.6P	3	>102400	176	<30
82	C4E9V-SP780-89.6P	3	>102400	<30	<30
89	C4E9V-SP788-89.6P & SP770-89.6P	3	819200	n/d	n/d
90	C4E9V-SP788-89.6P & SP770-89.6P	4	204800	n/d	n/d
91	C4E9V-SP780-89.6 & SP770-89.6	4	819200	n/d	n/d
92	C4E9V-SP780-89.6 & SP770-89.6	3	409600	n/d	n/d

*Assayed against HIVBAL in PBMC cultures.
n/d= not done.

Table 5

ELISA and neutralization assay summary of rabbit antisera raised against SHIV 89.6 envelope

G. Pig No.	Immunogen	Bleed No.	ELISA Titer	Neutralization in MT-2 (SHIV89.6)	Neutralization in MT-2 (SHIV-KB9)
7666	C4E9V-SP788-89.6P	4	65,536	<30	<30
7667	C4E9V-SP788-89.6P	4	65,536	<30	<30
7668	C4E9V-SP788-89.6P	4	65,536	<30	<30

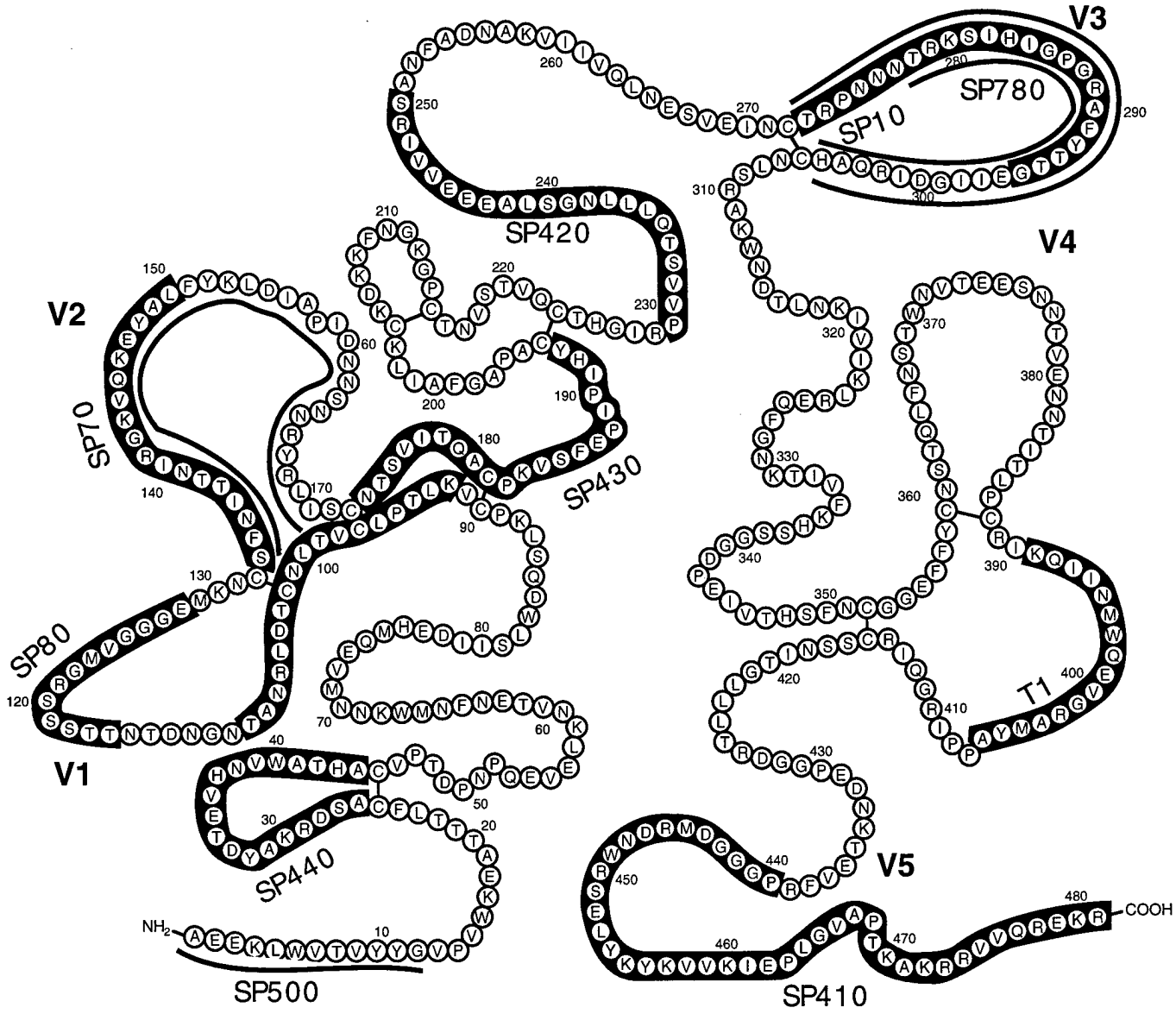

Figure 1. Schematic Representation of HIV Envolope Protein gp120. Highlighted regions represents that the peptides have been studied previously. New peptides (outlined) that are involved in HIV co-receptor binding are currently under investigation. (adapted from Leonard, et al., J.Biol. Chem. 265, 10373, 1990).

DAMD17-94-J-4467 PROGRESS REPORT - YEAR 4: STRUCTURAL AND FUNCTIONAL STUDIES OF EXPERIMENTAL HIV SYNTHETIC PEPTIDE IMMUNOGENS

Technical Aim 2: Structural Studies Using NMR

Investigator: L. D. Spicer, R. de Lorimier, H. M. Vu

Introduction

The objective of Technical Aim 2 is to study structural features of the immunogenic peptides which are the focus of this grant in order to establish structure-activity relationships. In this way we aim to understand on a molecular level important correlate characteristics of peptide HIV immunogens and use this information in design features of new immunogenic peptides. During the past year, exciting new results on the x-ray crystal structure of gp120 appeared (Kwong et al., 1998) which together with our own findings and recent reports of chemokine co-receptors CCR5 and CXCR4 (Choe et al., 1996) provide an exciting opportunity to extend our findings, and we plan to do so using results from our earlier efforts as a base. To date we have been engaged in determining conformational requirements needed to design peptide immunogens capable of inducing broadly cross-reactive anti-HIV neutralizing antibodies and anti-HIV T cell responses targeting HIV isolates grown either in T-cell lines or PBMC. Included in these studies are peptides already being characterized for immunogenic properties as well as new peptide constructs based on iterative evaluation of structure/function and design. Characterization and conformational modeling of the initial four peptides, C4-V3 RF, C4-V3 Can0, C4-V3 MN and C4-V3 EV91 are complete, and we have correlated those results with immunogenic data. Based on those findings, additional new peptides were designed to enhance reactivity, and each has been synthesized and characterized immunologically and by NMR. One of the mutations incorporated in the new peptides is now being used in more advanced peptide design and is being tested in Project 1 of this grant as described. A specific member of this promising peptide group, C4E9V-SP788, is being purified in NMR quantities along with synthetic peptide constructs from the extracellular domain of CCR5 for binding and structural studies based on promising activity and anticipated molecular assembly predicted from the co-crystal structures reported. Peptides based on gp41 B and T cell epitope sequences were also redesigned to enhance specific structural characteristics and were tested immunologically. We have amplified this project significantly based on positive earlier results and have extended our schedule to pursue this promising avenue to understanding the structural basis for function in experimental HIV synthetic peptide immunogens.

Body

A. The four C4-V3 peptides Can0, EV91, MN, and RF continue to be the focus for structural correlation in this study, and additional functional data on differential neutralizing capabilities in

rhesus monkeys has been obtained by Haynes and co-workers (Vu et al., submitted) to extend the comparisons. As stated in an earlier report, NMR assignments have been completed with respect to the proton resonances using a combination of 2-dimensional NMR experiments including COSY, TOCSY and NOESY. Conformational elements of the peptides have also been characterized from NOE connectivity maps and coupling constants. The RF and Can0 studies have been published (de Lorimier et al., 1994; Vu et al., 1996), and a manuscript describing EV91, MN and a comparison of all four peptides, has been submitted for publication (Vu et al., submitted). Results are summarized below.

Short- and medium-range NOEs were observed in all four peptides which indicated that the peptide conformations are not completely random; i.e. certain conformations are preferred. The C4 segment (also termed T1), which is identical in sequence in all four peptides, shows in each case a tendency to form nascent helical conformations, in the region from residue Asn5 to Tyr15. On the other hand the V3 (also called SP10) segments, which differ in sequence, show different preferred conformations in each peptide. The GPGX sequence at the tip of the V3 loop exhibits a tendency to form a reverse turn in each case, but the specific nature of the turns differentiates them. In Can0 a Type II β-turn is observed, but in RF, EV91 and MN a Type I β-turn tends to form. The RPXX sequence toward the C-terminal end of the V3 sequences tends to form a Type I β-turn in RF and MN, a Type II β-turn in EV91, and is in an extended conformation in Can0. Residues just preceding the GPGX turn in RF and Can0 are extended, but in EV91 form a second Type I turn. Consecutive turns like this at the tip of the V3 loop are also observed in the crystal structure of an MN epitope bound to the Fab fragment of a neutralizing antibody (Ghia et al., 1994). Residues immediately following the GPGX turn form nascent helices in EV91 and MN, but are extended in RF and Can0.

To further evaluate the organization of the peptides, particularly with respect to potentially important side chain orientations, the above-listed conformational elements suggested by NMR data were used to create models of the 13-residue region encompassing the GPGX motif at the tip of the V3 loop. Using molecular modeling software, the initial structures were optimized to avoid steric hindrance of the side chains and their surface potential energies were minimized before molecular dynamics calculations were performed. The refined models were compared with the published x-ray crystal structure of the antibody-bound MN V3 loop (Rini et al., 1993). An exposed relatively flat patch of apolar side chains immediately before the β turn of the bound MN peptide was reported in the crystal structure study and shown to interact extensively with residues in the antigen-binding pocket. This extended hydrophobic patch was observed in our modeling of Can0 and MN, but not in EV91 or RF. The RF model showed a disruption in the middle of this apolar surface due to protrusion of a charged side chain from a lysine residue. The corresponding hydrophobic region in the EV91 model was seen to be twisted as a result of the propensity of the proline in the sequence to promote a bend. Thus the EV91 model appeared more compact than its extended counterparts in Can0 and MN offering a more limited region of hydrophobic contact. The structural similarity of Can0 and MN, and their differences with EV91 and RF, parallels their immunogenicities in that Can0 and MN can induce more broadly cross-reactive antibodies (Haynes et al, 1995; Vu et al., submitted). We therefore suggest that the flat apolar surface observed in the x-ray structure of MN and in our Can0 model may act as a key conformational motif in inducing antibodies that are cross-reactive. Peptides EV91 and RF lack this motif, which may explain why they induce only type-specific antibodies.

B. Significant progress made in designing and characterizing peptide variants to enhance immunogenicity toward HIV during the past two years has also led to advanced design of larger peptides planned for future study. Designed variants of the C4-V3 RF peptide were particularly informative (de Lorimier et al., in preparation). As noted above, the C4 sequence, which contains T cell epitope called T1, exhibits helical conformations, but it has sequence homology to a region of IgA which is □ strand (de Lorimier et al., 1994). We previously hypothesized that this potential disparity in conformation might explain the absence of B-cell immunogenicity in the C4 portion of C4-V3 peptides, even though the C4 portion of native gp120 is antigenic. Thus we proposed to alter the sequence of C4 by single amino-acid substitutions that might reduce its helical tendency in solution and thus render it immunogenic for antibodies which recognize the C4 segment of gp120. Three variants of C4-V3 RF were designed and synthesized: Glu^{9}->Gly (E9G), Glu^{9}->Val (E9V), and Lys^{12}->Glu (K12E). In order to prevent peptide dimerization all three variants lacked the single cysteine which was present in the originally reported T1SP10RF(A) peptide (de Lorimier et al., 1994) at the junction between C4 and V3. A control peptide, C4-V3 RF, has the native C4 sequence but no cysteine residue. All four peptides (control and three variants) were studied for immunogenicity and conformation.

In terms of B-cell immunogenicity, none of the C4 sequence variants elicited detectable antibodies in mice which bind to C4 of intact gp120. One possible explanation for this finding is that the sequence alterations removed determinants important for recognition of native C4. Another is that more stringent constraints are required for C4, as a peptide, to be immunogenic for a B-cell response. In terms of T-cell immunogenicity, measured by antibody titer and splenocyte proliferation, the C4 variants induced very different responses. Variant E9V was a much more potent inducer of T-helper responses than the original C4 sequence, while K12E was significantly less immunogenic. Since C4 contains T1, an important MHC class II epitope, it may be expected that alteration of its sequence would affect function. In fact these results corroborate those of another study which examined T-cell immunogenicity in sequence variants of T1 (Boehncke et al., 1993).

To determine whether the designed conformations were exhibited by these peptides we studied them by NMR using the same methods previously employed for analyzing C4-V3 peptides. First resonance assignments were determined, then analysis of NOEs showed the position and relative population of nonrandom conformations in the peptide sequence. Peptide K12E yielded spectra with poor signal-to-noise, and the high viscosity of its solution suggested that it aggregates. To obtain usable spectra of K12E trifluoroethanol was added to 20%, and the temperature was raised to 45 C. Analysis of control peptide C4-V3 RF showed that its conformation was nearly identical to cysteine-containing T1SP10RF(A), including a helical propensity in C4. Variant E9V retained this helical character, while variant E9G lacked any NOEs indicative of a helical tendency. Variant K12E showed helical conformations in C4, but this may have been due to the presence of trifluoroethanol in the solution, which is known to induce helical conformations. Thus in at least one case, E9G, the designed solution conformation was attained.

The design element C4E9V has now been incorporated in new peptide constructs which comprise some of the targets for investigation in Project 1. One of these, now designated C4E9V-SP788, has shown promise for cross strain neutralization to SHIV 89.6 and contains the C-terminal extension of the V3 region to the end of the loop. We plan to study this peptide structurally pending further immunogenicity studies and in addition explore its binding along with

that of the other C4-V3 peptides to extracellular CCR5 epitopes. Complexation may very well stabilize the conformational flexibility of the peptides and reveal the details of co-receptor interaction expected from this domain in the crystal structures reported (Kwong et al., 1998).

Conclusions

The objectives of Technical Aim 2 regarding solution conformations of peptides have been achieved. Based on the insight gained from these results, two generations of design have been implemented to enhance immunogenicity and cross reactivity. The original four C4-V3 peptides have been characterized by NMR with respect to solution conformations. Molecular modeling of these peptides based on NMR results suggests a correlation between structural features and immunogenic properties. To test one hypothesis proposed from these studies, a set of peptides based on C4 sequence variants of C4-V3 RF was synthesized and characterized for solution conformations and immunogenicity. The results showed that conformational features can be designed with some success. In terms of design of function, it appears that in order to confer B-cell immunogenicity to T1 requires more that single amino-acid substitutions, but that T-cell immunogenicity can be profoundly affected by such substitutions. This latter feature is now being exploited with a new round of advanced design and structure-function testing. Initial results show enlarged cross-reactivity of at least one construct which is now being prepared for structural studies with and without co-receptor binding.

Studies for the extended funding period

The extension of this effort beyond the original four year period is especially opportune, based on 1) just published x-ray crystal structure studies of gp120 complexed with CD4 and co-receptor blocking antibody (Kwong et al., 1998) and 2) very recent immunological results reported in Technical Aim 1 as indicated in the above material. It is becoming clear from the crystal studies that functional interaction of gp120 involves the C4 sequence as part of a beta-sheet at the site of binding and most likely the variable V3 loop, which was truncated out of the crystallized coat protein, but is directly adjacent to the binding site. V3 has also been mapped biochemically as an interaction locus. The new peptides identified in Project 1 take advantage of this information, and one in particular, C4E9V-SP788 with a C-terminal extension, has already shown good promise with regard to primary isolates. Our plan is to investigate this peptide structurally in solution by NMR for correlation with activity and comparison with the conformational structures of the immunogenic peptides already determined in this project. In addition we will investigate other peptide constructs identified in Technical Aim 1 which show immunogenic activity, and we plan to explore structures of active peptide complexes from this and the original peptide group studied which bind to co-receptor CCR5.

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DAMD18-94-4467 PROGRESS REPORT - YEAR 4 (FINAL): STRUCTURAL AND FUNCTIONAL STUDIES OF EXPERIMENTAL HIV SYNTHETIC PEPTIDE IMMUNOGENS

Technical Aim 3: HLA-peptide interactions

Investigator: Donna D. Kostyu, Ph.D.

INTRODUCTION

The ability to elicit T cell responses to synthetic peptide immunogens depends, at the most basic level, on whether a peptide or seqences derived from it are presented effectively on an HLA class I or class II molecule. There are multiple variables to be considered: the sequence of the peptide, its length, ability to be processed, ability to bind to an HLA molecule, and then the stability of the peptide-HLA complex at 37C. The HLA molecules themselves are a considerable problem, as the number of alleles has grown to over 500, with striking differences in population distribution.

The aims of this project were to establish a simple, specific, predictive peptide-HLA binding assay and to define the restrictive alleles for the multivalent HIV peptide immunogens in this project. In doing so, we also developed a method for identifying class I subtypes by PCR and SSOP, analyzed the sites of polymorphism of 275 HLA-A, B and C alleles, explored the effect of HLA-A2 subtypes on peptide interactions, and applied an algorithm in order to identify target alleles in 15 populations. Overall, the goal was to establish basic rules for the design and testing of peptide immunogens.

BODY

A. PEPTIDE-HLA INTERACTIONS DEFINED BY A pH-DEPENDENT, CONFORMATIONAL BINDING ASSAY

Class I-peptide interactions have been defined in a peptide and beta-2-microglobulin (β2m) reconstitution assay, modifying the procedure originally reported by Zeh et al. (1). HLA-A and HLA-B molecules on C1R cells transfected with a single class I allele or reference T and B cell lines expressing multiple alleles were denatured with a pH3.1 buffer, then reconstituted in the presence of 50-100 uM peptide and 250 nM b2m. Loss and gain of conformation was monitored with a PE-labelled w6/32 monoclonal antibody for transfected cells and with allele-specific, biotin-labelled monoclonal antibodies and an avidin-FITC secondary reagent for cells expressing multiple class I alleles. The increase in mean fluorescent channel or intensity in cells incubated with b2m and peptide compared to cells incubated with β2m alone is used as an indication of peptide binding. This ratio allows comparison of results between assays. A ratio of 1.0 indicates

no binding, but in practice there is some fluctuation in fluorescence intensities and we have considered a binding ratio of 1.19 or more (mean + 3 standard deviations of replicate negative tubes) as positive binding. Controls included untreated cells exposed to an isotype control antibody (negative control), untreated cells exposed to the conformation-dependent monoclonal antibody (positive control), pH treated cells alone to demonstrate the effectiveness of pH stripping, and pH treated cells incubated with β2m alone. 0.02M monensin is included in each assay to block expression of new class I molecules during 37C incubations.

Over the time span of this project, this binding assay has proved to be a fundamental and precise technique. It has allowed us to characterize on and off rates of peptides, the binding of multiple peptides and truncated sequences, and the effect of HLA subtypes on peptide recognition. Because it is based on short binding times (<45 minutes) at 37C, we consider it more physiologic and more relevant than other types of binding assays that are done with soluble molecules over a 48 hour period at room temperature. Further, we are able to identify peptides that interact with cell membranes in an HLA-independent manner.

B. HLA CLASS I-RESTRICTED EPITOPES IN THE HIV V3 REGION

We have characterized the interaction of the V3 SP50 and AP10 peptides from the four multivalent peptide immunogens under study (T1SP10MN, T1SP10EV91, T1SP10RF, T1SP10Can0A) with multiple HLA-A and HLA-B alleles. In addition, we have included the V3 peptide p18 IIIB I10 and several non-HIV peptides with known HLA-A and B restrictions. Peptide sequences are provided in Table 1. The results of all binding assays are presented in Table 2, where each ratio is a mean of from 2 to as many as 10 independent experiments. Those ratios that we consider to be significant and likely to correspond to a CTL epitope are in boxes; weak binding of questionable significance is marked by dotted squares.

There are three HLA-B*0702 restricted sites:

(i)	V3 303-312	RPNYNKRKRI	SP50MN
		RPNNNTRKSI	SP50RF
		RPGNNTRKSI	SP50EV91
		RPHNNTRKSI	SP50Can0A
		RPGNNTRRGI	SP50Mal
		RPYQNTRQRT	SP50Eli

P2 (P) and P10 (I/T) are the likely anchor residues. Safrit and coworkers previously identified RPNNNTRKSI as an HLA-B7 restricted CTL site in two patients shortly after seroconversion (2).

(ii) V3 312-321 IPIGPGRAFI(ATS) AP10EV91

The 13mer AP10EV91 peptide interacts strongly with HLA-B*0702. However, based on reported anchor residues for HLA-B7, it seemed likely that the anchors were P2 (P) and P10 (I),

the same as above. We consequently synthesized a truncated peptide (IPIGPGRAFI) and found that it still binds to B*0702 molecules (Table 4), and thus may represent the minimal eptiope. Rubinstein and coworkers may have identified the same site when they observed CTL responses to the peptide KRIHIGPGRAFYT in HLA-B7+ vaccinees (3).

The AP10RF peptide (ITKGPGRVIYATG) also interacts with the HLA-B*0702 molecule, but such interactions are unstable at 37C and can only be detected in binding assays at room temperature. If the instability is caused by a poor fit with an HLA-B7 molecule, then the prediction would be that it is not capable of inducing a CD8+ T cell response. Alternatively, if the instability is due to inappropriate length or inability of a protease to trim, then this may also be a potential functional site.

(iii) V3 314-323 RGPGRAFVTI p18 IIIB

This peptide was originally synthesized because it contained an unusual HLA-A2.1 restricted site (4). However during testing, it was found to interact not only with HLA-A*0201, but also B*0701 and A*2402. Here, the usual reported anchor residues for HLA-B*0702 are not present.

There are two HLA-A9 (*2402) restricted sites:

(i) V3 312-320 IHIGPGRAF(YTTK) AP10MN
IHMGPGKAF(YTTG) AP10Can0A

The anchor residues are likely to be P2 (H) and P9 (F). A truncated MN sequence (IHIGPGRAF) (Table 4), binds to A*2402 as well, suggesting that the minimal epitope for both AP10MN and AP10Can0A is a 9mer. This site is similar to the A*2402-restricted CTL epitope IVIGPGRAF identified by Ikeda-Moore and coworkers in SF2 (5).

(ii) V3 314-323 RGPGRAFVTI p18 IIIB

This peptide binds strongly to A*2402, but lacks the usual anchor residues for A*2402.

In order to determine the lower limits of sensitivity in our experiments, we tested the A*0201 restricted p18 IIIB I10 peptide which is reported to have an extremely fast off-rate (2), and the human papillomavirus peptides E6 29-38 and E7 12-20, which are low-affinity binders (e.g. 400-500 nM) to HLA-A2 (6). All three peptides have been reported to induce CTLs. We found detectable binding of all three peptides to HLA-A*0201 at 37C, suggesting that any peptide which demonstrates a detectable interaction with an HLA-A or HLA-B molecule in our assay may represent a CTL target. Whether it does or not may depend upon factors that control antigen processing, presentation and immunodominance.

We found three examples of <u>very</u> low binding peptides, listed below, that are of questionable significance.

(i)	V3 303-312	SP50MN	RPNYNKRKRI	HLA-A*0101
(ii)	V3 303-312	SP50MN	RPNYNKRKRI	HLA-A*0201
(iii)	V3 314-323	AP10MN 3-12	IGPGRAFYTT	HLA-A*0201

The third peptide (AP10MN 3-12) targets the same site as the IIIB p18 I10 peptide (RGPGRAFVTI), which binds to HLA-A*0201 using P3, P5 and P7 anchor residues and which can induce HLA-A2 restricted CTLs (2). The AP10MN 3-12 peptide sequence reacts so poorly with A*0201, however, that it is probably not immunogenic. A detailed study of HLA-A2 subtypes is discussed later.

Some proteolytic processing of peptides occurs during our binding assay. The AP10EV91 peptide (IPIGPGRAFIATS) and the AP10MN peptide (IHIGPGRAFYTTK) bind to HLA-B*0702 and HLA-A*2402 respectively, even though these peptides are 13 amino acids long. We synthesized truncated peptides that corresponded to what we would predict the minimal epitope to be, based on published anchor residues. The minimal recognized epitope was a 10 mer for AP10EV91 (IPIGPGRAFI) and a 9 mer for AP10MN (IHIGPGRAF) (Table 4). While it is possible that 13 residue peptides might bind to class I molecules by bulging in the middle, it is more likely that a protease in our assay is clipping off the carboxyterminal end. Even though our assay is performed in plain media, there could be cell-surface proteases (which may be activated during pH 3.1 treatment to remove existing peptides and β2m) or some carryover of proteases in the fetal calf serum in which cells are grown. Proteolytic cleavage by fetal-calf serum components was shown previously by Kozlowski and coworkers (7, 8).

Although most of our work has concentrated on four peptide immunogens, the IIIB p18 V3 sequence that was added as a control turned out to be quite interesting. The 15 amino acid p18 IIIB peptide is considered to be promiscuous because it contains HLA-A2, A3, A11, and multiple H-2 restricted epitopes (4, 9). The truncated version (p18 IIIB I10) contains the minimal A*0201 recognition site (4). When we tested this shorter peptide, it bound not only to A*0201, but also to A*2402 and B*0702. Thus the 15 amino acid p18 IIIB peptide actually contains five potential CTL epitopes: A*0201, A3, A11, A*2402 and B7*0702.

C. UNUSUAL REACTIVITY OF THE T1 PEPTIDE

The T1 peptide has long been considered to contain a Th epitope. Before setting up a class II peptide binding assay to define interactions of T1 and HLA-DR or DQ, we included T1 in class I binding assays and found anomalous binding. The T1 peptide bound to pH treated and untreated cells regardless of HLA type and in a β2m-independent manner. It bound to the class II negative HSB cell line and to class I and class II negative human red cells (Table 5). It often spontaneously aggregated cells by itself. Once attached to the cell surface, it was able to bind avidin-FITC in a non-specific manner, which allowed us to monitor its activity. Increasing

concentrations of T1 continued to bind, with no plateau. We believe that the T1 peptide aggregates or pools in water, accounting for the intense fluorescent signals and cell agglutination. Functional activity was localized to the amino terminal end of the T1 peptide, as the truncated T1.1 (KQIINMWQEV) and T1.2 (IINMWQEVGKA) peptides were still capable of binding to cells, although to a lesser degree than the whole 16mer. Removal of the four amino-terminal residues in truncated peptides (T1.3-T1.6) eliminated cell-surface reactivity (Table 5).

During the study of these multivalent peptide immunogens, amino acid substitutions designed to enhance or minimize alpha helical properties were incorporated in the T1 region of the 39 residue peptides. The substitution of an E to V at position 9 (T1SP10RF(A)E9V) enhanced antibody responses, while a K to E substitution at position 12 (T1SP10RF(A)K12E) was associated with a loss of antibody responses. We synthesized T1 mutant peptides, T1 E9V and T1 K12E, and found that the T1 E9V peptide aggregated and bound cells almost as well as T1 by itself. The T1 K12E peptide lost nearly all of this activity (Table 5). There are two possible explanations. The T1 peptide might spontaneously adapt an extended, aggregated conformation in water, capable of binding to and agglutinating cells in a charge-related, but nonspecific manner. The alternative explanation is that the sequence KQIINMWQEVGKA contains or mimics a heparan-sulfate or other cell surface binding site.

In our initial binding assays, we included the 39mer peptides as negative controls. We were surprised to see binding of T1SP10MN(A) to several cell lines. This binding turned out to be HLA-independent. Further testing indicated that the peptide bound to normal, untreated B and T cell lines by itself, but that the shift in fluorescence was very small and probably would not be noticed under normal conditions. It is detectable on pH treated cells where the background fluorescence is very low and where the avidin-FITC used as a secondary reagent may bind nonspecifically to the T1 region of the 39mer peptide. We believe that the T1SP10MN(A) peptide allows some residual conformation of the T1 region, such that non-specific binding to cells via the T1 region can still occur at a very low level. It is possible that the sequence of the V3 peptide affects the ability of the T1 region to spontaneously aggregate. The T1SP10MN(A) and T1SP10RF(A) E9V peptides maintain some of the conformational characteristics of the T1 peptide itself, where as the T1SP10RF(A) K12E does not. This further suggests that the immunogenicity of the 39mer peptides may in part be due to the ability of the T1 region to spontaneously form aggregates or specific complexes, bind cells, and enter a class I or class II pathway. We tested two other reported Th epitopes (TT830 and p18 IIIB) to determine if this ability to stick to cells was a general property of Th epitopes. It was not.

D. EXTENT OF POLYMORPHISM AT HLA-A, HLA-B AND HLA-C

We analyzed a database of 275 HLA-A, HLA-B and HLA-C alleles in order to identify the exact sites of polymorphic residues and probable impact on peptide binding or interaction with other molecules (10). Modeling was done using the programs MOLSCRIPT and RasMol, in collaboration with Dr. Linda Hannick. We found that polymorphism was spread throughout the molecule, e.g. 47/90 residues were polymorphic in the alpha 1 domain, 41/92 residues in the alpha 2 domain, 21/92 in the alpha 3 domain, 20/40 in the transmembrane region, and 7/28 in the

cytoplasmic region. These sites have been summarized in Tables 6-8. Polymorphic residues inside the peptide binding groove and along the alpha helices may affect peptide binding and stability. There is, however, polymorphism in other areas that will not have any effect on peptide binding, but may affect interactions with b2m, chaperones in the endoplasmic reticulum, CD8, or NK receptors. Thus, the design of peptides that will elicit CD8+ T cell responses might depend upon whether a particular class I molecule will not only bind a peptide with sufficient affinity and stability, but also whether all subtypes of that allele will bind and present in a similar manner, and if all give rise to efficient production of CTLs.

The increasing number of alleles is a serious concern for vaccine development. The differences within an allele family may be very small. For example, HLA-A*0101 and A*0102 differ by two amino acids in the alpha 1 domain. In contrast, the HLA-A2 family is large and diverse. Some serologically unique alleles such as HLA-B*0702, *3501, B51, B*5301 and B*5401 appear to share common sequences around peptide binding pockets and may have overlapping peptide binding patterns (11). It has been suggested that peptides using these pockets will have broader population coverage.

E. IDENTIFICATION OF TARGET ALLELES IN CAUCASIAN, BLACK, AND ASIAN POPULATIONS

Given the large and still growing number of alleles and the skewed distributions across populations, we applied an algorithm to identify the predominant alleles in 5 Caucasian populations (French, Spanish, USA, Canadian, Brazilian), 5 Black populations (Capetown, South Africa, USA, Brazilian, San Bushmen) and 5 Asian populations (Japanese, Korean, Northern Han Chinese, Southern Han Chinese, and Thai). The algorithm produces cumulative frequencies of HLA-A (Table 9) and HLA-B (Table 10) alleles. This work was done in collaboration with Dr. Deborah Dawson.

Our analysis shows that HLA-A may be a better candidate than HLA-B for eliciting responses in large numbers of individuals, as there are fewer alleles and consequently greater coverage. For HLA-A, 80% coverage can be obtained with only two alleles in several Asian populations due to the high frequency of HLA-A2, A24 and A11. In Caucasian populations, three alleles (A1, A2, A3) suffice in US and Canadian populations, with A24 added in the French and A29 in the Spanish. Increased heterogeneity is seen in the Brazilian Caucasian population and several Black populations, pointing out the potential difficulties in admixed populations. HLA-A30, A28 and A23 are common alleles in Black populations, but are rarely observed in Caucasians. Only HLA-A2 is seen at high frequency in all ethnic groups examined here.

The problem with peptides restricted to HLA-B alleles is that there are more HLA-B alleles and these are found at lower allele frequencies. Three alleles are found in approximately 80% of individuals in South African and San Bushmen populations, and four alleles are observed in the Southern Han Chinese population. For the remaining ethnic groups, the number of alleles required for 80% coverage grows rapidly, to six to eight and even ten alleles. No commonly

shared, high frequency allele is observed at HLA-B for all ethnic groups. Moreover, the targeted B alleles differ widely from Caucasians to Blacks to Asians. Thus, HLA-B restricted CTL epitopes may be more problematic for widespread population coverage.

F. MOLECULAR DEFINITION OF HLA-A AND HLA-B ALLELES BY PCR AND SSOP

We designed and tested more than 20 primer sets and 85 probes for high resolution HLA-A and HLA-B typing by ELISA. The currently used probes are listed in Table 11. This provides for the identification of polymorphic residues in both the alpha 1 and alpha 2 domains of each molecule. The specificity and segregation of these probes was confirmed in rheumatoid arthritis families.

G. EFFECT OF HLA-A2 POLYMORPHISM ON PEPTIDE RESPONSES

The distribution of alleles such as HLA-A1, A2, and B7 among ethnic groups is well documented. However, the distribution of allele subtypes is less understood. HLA-A2, for example, is found in nearly half of the world's population. There are common subtypes. HLA-A*0201 constitutes almost 100% of the A2 alleles found in Caucasians (12) and is present in nearly all other populations at lower levels. HLA-A*0202 and A*0205 are restricted to Blacks, and *0203, *0206, and *0207 to Asians. In order to test the influence of A2 subtypes on binding of several V3 peptides, we tested SP50MN, the A*0201 restricted flu matrix peptide, two human papillomavirus peptides E6 26-38 and E7 12-20, and the p18 IIIB I10 peptide for their ability to interact with seven HLA-A2 subtypes (Table 12) . No peptide was capable of binding strongly to all of the A2 subtypes. The flu peptide was most reactive, binding to A*0201, *0202, *0204, *0205, *0206/0207 and *0217. Binding to A*0203 weak. In contrast, the other five peptides were able to bind to A*0201, but only infrequently and at very low levels to other subtypes. Thus, the flu peptide may be able to induce CTLs in nearly all populations, but other peptides may have limited responses in non-Caucasian ethnic groups. It is interesting that the flu peptide uses P2 and P9 as anchors (13), whereas the HIV IIIB p18 I10 peptide uses P3, P7 and P10 anchors (4).

Our results point out several things. The A*0201 is a flexible molecule, capable of accommodating peptides with different anchors. This does not appear to be true for A*0202, A*0203, A*0205 and A*0206/0207 molecules, all common subtypes in non-Caucasian populations. Therefore, some peptides that are immunogenic in Caucasians because they bind to A*0201 molecules may nevertheless be poor immunogens in non-Caucasian populations that carry other HLA-A2 allele subtypes. This should be testable in vitro. Incomplete knowledge of HLA-A2 subtypes in populations may also complicate or mask the specificity of CTLs.

We have identified HLA-B*0702 and A*2402 restricted sites in the multivalent peptide immunogens studied here. There are at least 6 HLA-B7 subtypes and 10 HLA-A24 subtypes, but little or no information on the frequency of these subtypes in different ethnic groups.

Consequently, it is not possible to predict if these subtypes are relevant. For HLA-A24, for example, the subtypes differ at any of 16 positions within the alpha 1 and alpha 2 domains. Sette and coworkers have described several peptides that can be presented by multiple HLA molecules, including some peptides that can bind to HLA-B7, B35 and B51 (the B7 supertype) (11). We tested the HLA-B*0702 restricted SP50 and AP10 peptides to determine if they were equally reactive. They failed to bind to B35 and B51 molecules.

CONCLUSIONS

The ability to design a peptide vaccine that elicits CD8+ T cell responses is constrained by the striking polymorphism at HLA-A and HLA-B, the occurrence of multiple subtypes of alleles (not all of which will bind the same set of peptides), and ethnic distribution of alleles. How much of a problem these will be, and whether it will be possible to circumvent them, is not yet clear.

High resolution class I typing and identification of subtypes is still difficult, time intensive, and expensive. As a result, comprehensive population data is not available for most DNA-defined allele subtypes. Some inbred and homogeneous ethnic groups (such as those found in Africa, or Native Americans) may carry only one or two allele subtypes at high frequencies, such that serologic and molecular HLA typing is straightforward. In contrast, ethnic groups of mixed background (such as the Brazilian populations) contain multiple HLA-A and B alleles, at low frequency, and with the possibility of multiple allele subtypes. Thus, extrapolation across ethnic groups is risky and predictions should be backed by laboratory testing of allele and peptide.

Despite an ability to define peptide interactions with single HLA molecules in vitro, predicting CD8+ T cell responses in vivo is much more difficult. Are the number of CD8+ precursor cells the same for each peptide immunogen? Does binding of a peptide to several different class I alleles result in identical CD8+ T cell function? Could some low-affinity peptide-HLA interactions generate incomplete T cell signals and induce tolerance or anergy? Are there dominant epitopes and does dominance correlate with binding affinity or number of responding T cells or other factors such as proteasome and TAP function? Is there a difference in peptides presented on HLA-A vs. HLA-B vs. HLA-C? Thus, defining peptide-HLA class I interactions is really just a beginning. We may be able to predict what peptides should be presented in a suitable way for T cell recognition. The question now is what sort of T cell responses are actually observed.

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Table 1

List of peptides sequences

(C4: residues from 428; V3: residues from 302)

Peptide	Sequence
T1Sp10MN(A)	K Q I I N M W Q E V G K A M Y A T R P N Y N K R K R I H I G P G R A F Y T T K
T1Sp10RF(A)	- N - T - - S - T K - - - - V I - A - G
T1Sp10EV91(A)	- - - - - - - - - - - - - - - - - - - G N - T - - S - P - - - - - - - I A - S
T1Sp10CANOA(A)	- - - - - - - - - - - - - - - - - - - H N - T - - S - - M - - - K - - - - - G
T1Sp10RF(A) K12E	- - - - - - - - - - - E - - - - - - - - N - T - - S - T K - - - - V I - A - G
T1SP10RF(A) E9V	- - - - - - - - V - - - - - - - - - - - N - T - - S - T K - - - - V I - A - G
T1SP10RF(A) E9G	- - - - - - - - G - - - - - - - - - - - N - T - - S - T K - - - - V I - A - G
T1	K Q I I N M W Q E V G K A M Y A
T1.1	K Q I I N M W Q E V
T1.2	I I N M W Q E V G K A
T1.3	N M W Q E V G K A M Y
T1.4	N M W Q E V G K A
T1.5	W Q E V G K A M Y
T1.6	E V G K A M Y A
T1 E9V	K Q I I N M W Q V V G K A M Y A
T1 K12E	K Q I I N M W Q E V G E A M Y A
SP50 MN	R P N Y N K R K R I
SP50 RF	R P N N N T R K S I
SP50 EV91	R P G N N T R K S I
SP50 CANOA	R P H N N T R K S I
SP50 MAL	R P G N N T R R G I
SP50 ELI	R P Y Q N T R Q R T
AP10 MN	I H I G P G R A F Y T T K
AP10 MN (1-9)	I H I G P G R A F
AP10MN (3-12)	I G P G R A F Y T T
AP10 RF	I T K G P G R V I Y A T G
AP10 EV91	I P I G P G R A F I A T S
AP10 EV91 (1-10)	I P I G P G R A F I
AP10 CANOA	I H M G P G K A F Y T T G
AP10 CANOB	I N I G P G R A F Y T T G
HIV IIIB p18 I10	R G P G R A F V T I
FLU 58-66	G I L G F V F T L
Mage 3	E V D P I G H L Y
HPV E6 29-38	T I H D I I L E C V
HPV E7 12-20	M L D L Q P E T T

Table 2

Summary of peptide binding assays

Peptides:	Alleles									
	A*0101	A*0201	A*0301	A*2402	A*68	A*3101	B*0702	B*0801	B*4402	B*5101
V3 SP50MN	1.10	1.08	1.02	0.99	1.05	0.99	1.68	1.00	1.02	0.93
V3 SP50RF	0.91	0.96	1.02	0.88	0.99	0.93	1.75	1.01	1.03	0.97
V3 SP50EV91	1.00	1.02	0.99	0.98	0.99	0.88	1.70	1.00	1.04	0.96
V3 SP50CANOA	0.96	0.88	0.97	0.94	0.96	1.00	nt	0.97	1.04	0.91
V3 SP50MAL	0.88	0.99	1.00	0.96	0.99	0.91	1.76	0.98	0.99	1.05
V3 SP50ELI	1.00	1.00	0.99	0.96	0.99	0.93	1.73	1.00	1.03	100
V3 AP10MN	0.96	0.98	1.00	1.60	1.00	0.87	1.02	0.96	0.95	0.88
V3 AP10RF	0.96	0.98	0.99	0.99	0.98	0.96	1.13 *	1.04	1.00	0.90
V3 AP10EV91	0.98	1.00	0.96	0.91	0.99	0.93	1.60	1.04	1.00	1.00
V3 AP10CANOA(A)	1.01	1.00	0.97	1.52	0.99	0.92	1.00	0.93	1.01	0.92
V3 AP10CANOB(A)	0.93	0.97	0.93	0.91	1.00	0.97	0.99	0.89	1.04	0.92
V3 AP10.1 (p18 IIIB)	nt	1.27	nt	1.53	nt	nt	1.75	nt	nt	1.00
V3 AP10.2 (MN)	nt	1.10	nt	0.97	nt	nt	1.01	nt	nt	0.95
FLU MATRIX	1.06	1.57	0.90	nd	nt	nt	0.93	0.87	0.91	0.96
MAGE3	2.67	0.98	1.00	1.01	nt	nt	1.03	nt	0.95	0.93
HPV E6 29-38		1.25								
HPV E7 12-20		1.21								

Numbers represent a ratio of the mean fluorescent intensity (MFI) of cells + peptide + b2m/MFI of cells + b2m alone. Ratios >1.19 are considered significant. Ratios between 1.10 and 1.19 are weak and are of uncertain relevance

* Temperature-dependent

Table 3

Potential CTL epitopes in V3 regions, defined by a peptide-HLA class I binding assay

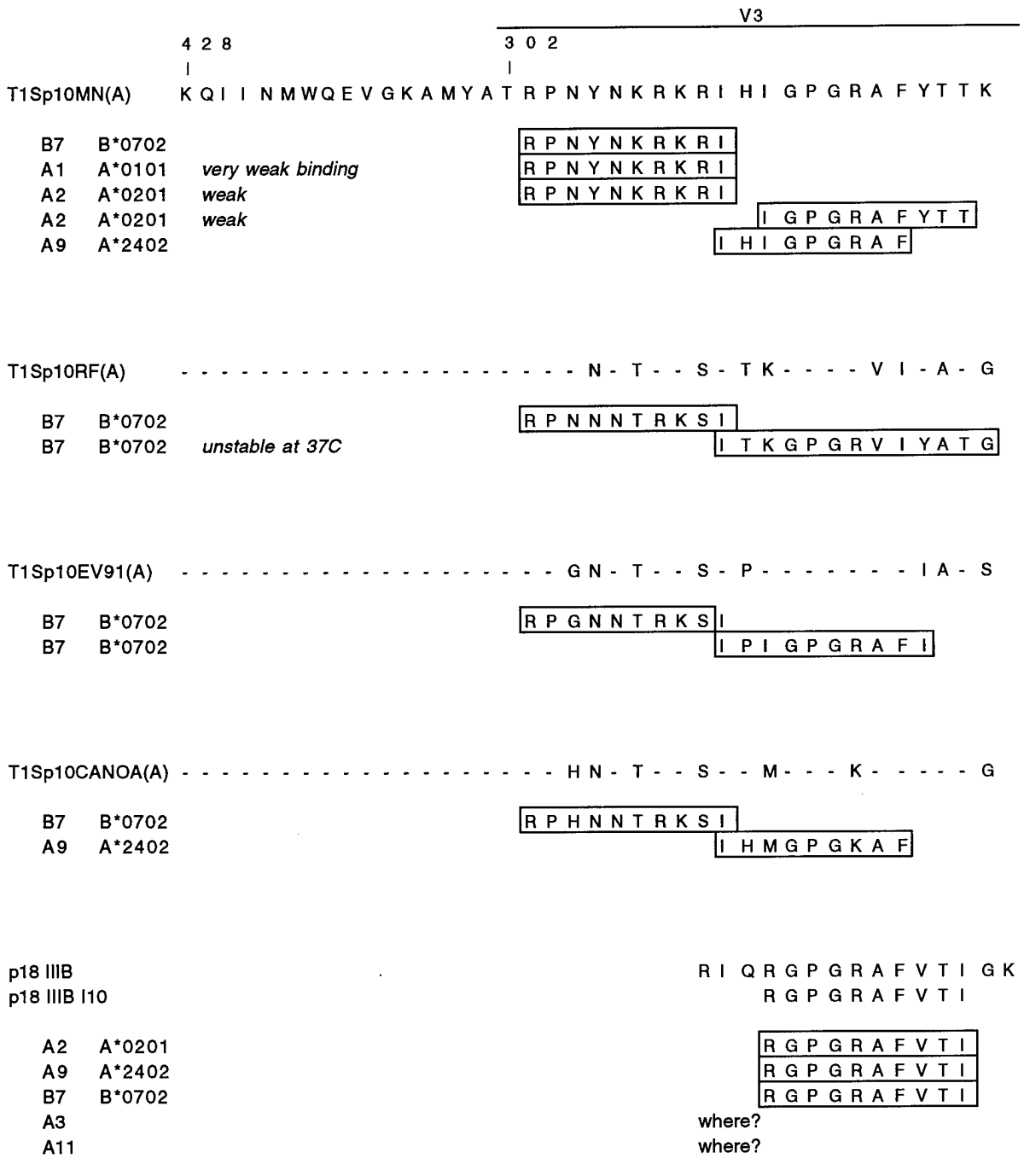

Table 4
Minimal epitopes for HLA-A9 (A*2402) and AP10MN
and HLA-B7 (B*0702) and AP10EV91

peptide	sequence	allele	binding ratio
AP10MN	IHIGPGRAFYTTK	A*2402	1.37
AP10MN (1-9)	IHIGPGRAF	A*2402	1.24
Mage (negative control)		A*2402	.97
AP10EV91	IPIGPGRAFIATS	B*0702	1.60
AP10EV91 (1-10)	IPIGPGRAFI	B*0702	1.32
Mage (negative control)		B*0702	.91

TABLE 5

T1 PEPTIDE INTERACTIONS WITH LYMPHOBLASTOID CELL LINES AND RED BLOOD CELLS

Expt	Cells	peptide	sequence	avidin-FITC	MFI	SD
1	LCL	none		+	3.14	.20
		T1SP10MN(A)		+	6.00	.18
		T1SP10RF(A) E9G		+	6.32	.81
		T1SP10RF(A) K12E		+	2.80	.11
		SP50MAL		+	2.47	.18
		MAGE3		+	2.80	.28
		T1		+	215.00	
2	red blood cells	none		+	2	
		T1SP10MN(A)		+	11	
		T1SP10RF(A)		+	5	
		T1SP10RF(A) E9G		+	12	
		T1SP10RF(A) E9V		+	74	
		T1SP10RF(A) K12E		+	4	
		SP50MAL		+	2	
		Mage3		+	3	
		T1	KQIINMWQEVGKAMYA	+	1310	
		T1 E9V	KQIINMWQVVGKAMYA	+	1687	
		T1 K12E	KQIINMWQEVGEAMYA	+	120	
3	LCL	none		+	6	
		T1	KQIINMWQEVGKAMYA	+	2406	
		T1.1	KQIINMWQEV	+	445	
		T1.2	IINMWQEVGKA	+	663	
		T1.3	NMWQEVGKAMYA	+	7	
		T1.4	NMWQEVGKA	+	5	
		T1.5	WQEVGKAMYA	+	7	
		T1.6	EVGKAMYA	+	6	

TABLE 6 POLYMORPHIC RESIDUES AT HLA-A

		alpha 1	alpha 2	alpha 3	TM	C
		1 1 1 3 3 4 4 5 6 6 6 6 6 7 7 7 7 7 7 8 8 8 8 9	9 9 9 0 0 0 0 1 1 1 2 4 4 4 4 5 5 5 5 5 6 6 6 6 7	8 8 9 9 0 3 4 4 5 5 6	7 8 8 8 9 9 9 0 0 1 1	1 2 3
		3 9 2 7 9 1 5 3 4 6 2 3 5 6 7 0 3 4 6 7 9 0 1 2 3 0	5 7 9 2 5 7 9 4 5 6 7 2 4 5 9 0 1 2 6 8 1 3 6 7 1	4 6 3 4 7 6 5 6 3 5 8	6 2 3 8 4 8 9 0 8 1 2	5 2 5
		H Y V R E T R P R G R E Q I V N T D E S R N L R G A	L R Y D P G L D Q Y N I Q R A A R V L A E T E W Y	P K P I G A A A E Q K	P I P V L V V I M R K	G S V
A1	A*0101	- F - - - - - Q K - Q - R N M H - - A N G T - - - D	I I - - - - F R - D - - K - - V H A R V - R D G -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
	A*0102	- S - S - - - Q K - Q - R N M H - - A N G T - - - D	I I - - - - F R - D - - K - - V H A R V - R D G -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
A2	A*0201	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0202	- F - - - - - R - - G - R K - H - H V D G T - - - -	- - - - S W F H - - K T K H - - H - W - - - - - -	A - A V S - - - Q - -		
	A*0203	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K T K H T - H E W - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0204	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V M - - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0205	- - - - - - - R - - G - R K - H - H V D G T - - - -	- - - - S W F H - - K T K H - - H - W - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0206	- - - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0207	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - C - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0208	- - - - - - - R - - G - R N - H - H V D G T - - - -	- - - - S W F H - - K T K H - - H - W - - - - - -	A - A V S - - - Q - -		
	A*0209	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K T K H - - H - - - - - - - -	A - A V S E - - Q - -		
	A*0210	- - - - - - - Q - - G - R K - H - H V D G T - - - -	V - F - S - F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0211	- F - - - - - Q - - G - R K - H I - V D G T - - - -	V - - - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0212	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K T K H - - H - Q - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0213	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K T K H - - H E Q - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0214	- - - - - - - R - - G - R K - H - H V D G T - - - -	- - - - S W F H - - K T K H - - H - - - - - - - -			
	A*0215N	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - C - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0216	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K T K H - - H - - - - E - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0217	- F - - - - - Q - - G - R K - H - H V D G T - - - -	- M F - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
	A*0219	- F - - - - - Q - - G - R K - H - H V D G T - - - -	V - - - S W F H - - K - K - - H - Q - - - D G -			
	A*0220	- F - - - - - Q - - G - R N - H - H V D G T - - - -	V - - - S W F H - - K T K H - - H - - - - - - - -			
A3	A*0301	- F - - - - - Q - - Q - R N - Q - - V D G T - - - -	I I - - S - F R - D - - K - - - H E - - D - - - -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
	A*0302	- F - - - - - Q - - Q - R N - Q - - V D G T - - - -	I I - - S - F R - D - - K - - - H - Q - D - - - -	- - - - - - - - - - -		
	A*0303	- F - - - - - Q - - Q - R N - Q - - V D G T - - - -	I I - . S - F R - D - - K - - - H E - - D - - - -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
A11	A*1101	- - - - - - - Q - - Q - R N - Q - - V D G T - - - D	I I - - - - F R - D - - K - - - H A Q - - R - - -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
	A*1102	- - - - K - - Q - - Q - R N - Q - - V D G T - - - D	I I - - - - F R - D - - K - - - H A Q - - R - - -	- - - - - - - - - - -		
	A*1103	- - - - - - - Q - - Q - R N - Q - - V D G T - - - D	I I - - - - F R - D - - K - - - - E Q - - R - - -			
	A*1104	- - - - - - - Q - - Q - R N - Q - - V D G T - - - D	I I - - - - F R - D - - K - - - H A Q - - - - - -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
A9	A*2301	- S - - - - - Q - - E - G K - H - - - N - I A L R -	- M F - S - F H - - K - - - - - - - - - D G -	- - - - - - - - - - -	- V H I - - I T - - N	D - -
	A*2402	- S - - - - - Q - - E - G K - H - - - N - I A L R -	- M F - S - F H - - K - K - - - H - Q - - - D G -	- - - - - - - - - - -	- V - I - - I T - - N	D - -
	A*2403	- S - - - - - Q - - E - G K - H - - - N - I A L R -	- M F - S - F H - - K - K - - - H - Q - - - - - -	- - - - - - - - - - -	- V - I - - I T - - N	D - -
	A*2404	- S - - - - - Q - - E - G K - H - - A N G T - - - -	- M F - S - F H - - K - K - - - H - Q - - - D G -	- - - - - - - - - - -	- V - I - - I T - - N	D - -
	A*2405	- S - - - - - Q - - E - G K - H - - - N - I A L R -	- M F - S - F H - - K - - - - - H - Q - - - D G -			
	A*2406	- S - - - - - Q - - E - G K - H - - - N - I A L R -	- M F - S - F H - - K - K - - - H - W - - - D G -			
	A*2407	- S - - - - - Q - - E - G K - Q - - - N - I A L R -	- M F - S - F H - - K - K - - - H - Q - - - D G -	- - - - - - - - - - -	- V - I - - I T - - N	D - -
	A*2408	Q S - - - - - Q - - G - R K - H - - - N - I A L R -	- M F - S - F H - - K - K - - - H - Q - - - D G -	- - - - - - - - - - -	- V - I - - I T - - N	D - -
	A*2409	- S - - - - - Q - - E - G K - H - - - N - I A L R -	- M F - S - F H - - K - K - - - H - Q - - - D G -	- - - - - - - - - - -	- V - I - - I T - - N	D - -
	A*2410	- S - - - - - Q - - E - G K - H - - - N - I A L R -	- M F - S - F H - - K - K - - - H - Q - - R - - -			
	A*2414	- S - - - - - Q - - E - G K - H - - - N - I A L R -	R - - - S W F H - - K - K - - - H - - - - - D G -			
A10	A*2501	- - - - - - - Q - - - N R N - H - - - - - I A L R D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*2502	- - - - - - - Q - - - N R N - Q - - - - - I A L R D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*2601	- - - - - - - Q - - - N R N - H - - A N G T - - - D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*2602	- - - - - - - Q - - - N R N - H - - A N G T - - - D	I - - - - - F Q - N - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*2603	- - - - - - - Q - - - N R N - H - H V D G T - - - D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*2604	- - - - - - - Q - - - N R N - H - - A N G T - - - D	I - - - - - F Q - D - - - - T - H E W - - L - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*2605	- - - - - - - Q - - - N R N - H - - - N G T - - - D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*2606	- - - - - - - Q - - - N R N - H - H V D G T - - - D	I - - - - - F Q R D - - - - T - H E W - - R - - -			
	A*2607	- - - - - - - Q - - G - R K - H - - A N G T - - - D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
A29	A*2901	- T - - - - - Q - - L Q R N - Q - - A N G T - - - -	I M - H S - F R - D - - - - - - - - - - - - - - -	A - A V S - - S Q - -	- - - I F - F A R - -	D F M
	A*2902	- T - - - - - Q - - L Q R N - Q - - A N G T - - - -	I M - - S - F R - D - - - - - - - - - - - - - - -	A - A V S - - S Q - -	- - - I F - F A R - -	D - M
A30	A*3001	- S - S - - - Q - R Q - R N - Q - - V D G T - - - -	I I - - S - F E - H - - - - - - - W - - - - - - -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
	A*3002	- S - S - - - Q - R Q - R N - H - - - N G T - - - -	I I - - S - F E - H - - - - - - R - - - - - - - -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
	A*3003	- S - S - - - Q - - Q - R N - H - - - N G T - - - -	I I - - S - F E - H - - - - - - R - - - - - - - -	- - - - - - - - - - -		
	A*3004	- S - S - - - Q - R Q - R N - H - - - N G T - - - -	I I - - S - F E - H - - - - - - H - W - - - - - -			
A31	A*31012	- T - - - - - Q - R Q - R N - H I - V D G T - - - -	I M - - S - F Q - D - - - - - - - - - - - - - - -	- - A V S - - S Q - -	- - - I F - F A R - -	D - M
A32	A*3201	- F - - - - - Q - - Q - R N - H - - - - - I A L R -	I M - - - - - Q - D - - - - - - - - - - - - - - -	A - A V S - - S Q - -	- - - I F M F A R - -	D - M
	A*3202	- F - - - - - Q - - Q - R N - H - - - - - I A L R -	I M - - - - - Q - D - - - - - - H - Q - - - - - -	A - A V S - - S Q - -	- - - I F M F A R - -	D - M
A33	A*3301	- T - - - - - Q - - - N R N - H I - V D G T - - - -	I M - - S - F Q - D - - - - - - - - - - - - - - H -	R A V S - - S Q - -	- - - I F - F A R - -	D F M
	A*3303	- T - - - - - Q - - - N R N - H I - V D G T - - - -	I M - - S - F Q - D - - - - - - - - - - - - - - -	- - A V S - - S Q - -	- - - I F - F A R - -	D - M
A34	A*3401	- - - - - - - Q - - - N R K - Q - - V D G T - - - D	I - - - - - F Q - D - - - - T - H E W - - - - - -	A - A V S - - S Q - -	- - - L F - I A - - -	D - M
	A*3402	- - - - - - - Q - - - N R N - Q - - V D G T - - - D	I I - - - S - F R - D - - - - T - H E - - - - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
A36	A*3601	- F - - - - - Q K - Q - R N M H - - A N G T - - - D	I I - - - - F R - D - - K - - V H A R V - - - - -	- - - - - - - - - - -	L - - I - - I T - - -	D T -
A43	A*4301	- - - - - - - Q - - L Q R N - H - - A N G T - - - D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
A66	A*6601	- - - - - - - Q - - - N R N - Q - - V D G T - - - D	I - - - - - F Q - D - - - - T - H E W - - R - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*6602	- - - - - - - Q - - - N R N - Q - - V D G T - - - -	I - - - - - F Q - D - - - - T - H E W - - E - - -	A - A V S - - S Q - -	- - - I F - I A - - -	D - M
	A*6603	- - - - - - - Q - - - N R N - H - - V D G T - - - -	I - - - - - F Q - D - - - - T - H E W - - E - - -			
A28	A*68011	- - - - - - - Q - - - N R N - Q - - V D G T - - - -	I M - - S - F R - D K T K H - - H - W - - - - - -	A - A V S - V - Q - -	- - - I F - I T - - -	D - -
	A*68012	- - - - - - - Q - - - N R N - Q - - V D G T - - - -	I M - - S - F R - D K T K H - - H - W - - - - - -	A - A V S - V - Q - -	- - - I F - I T - - -	D - -
	A*6802	- - M - - - - Q - - - N R N - Q - - V D G T - - - -	I - - - - - F H - - K T K H - - H - W - - - - - -	A - A V S - V - Q - -	- - - I F - I T - - -	D - -
	A*6901	- - - - - - - Q - - - N R N - Q - - V D G T - - - -	V - - - S W F H - - K T K H - - H - - - - - - - -	A - A V S - - - Q - -	- - - I F - I T - - -	D - -
A74	A*7401	- F - - - - - Q - - Q - R N - H - - V D G T - - - -	I M - - - - - Q - D - - - - - - - - - - - - - - -	A - A V S - - S Q - -	- - - I F M F A R - -	D - M
	A*7402	- F - - - - - Q - - Q - R N - H - - V D G T - - - -	I M - - - - - Q - D - - - - - - - - - - - - - - -			
A80	A*8001	- F - - - S Q Q - E E - R N - H - N A N G T - - - D	I I - - S - F R - D - - K - - - - R - - - E D G -	- - - - S - - - K K E	- - - I - - - I A - K -	V - -

TABLE 7 POLYMORPHIC RESIDUES AT HLA-B

			alpha 1	alpha 2	alpha 3	TM	C
			1 1 1 2 3 3 4 4 4 5 5 6 6 6 6 6 6 7 7 7 7 8 8 8 8 9	9 9 9 9 0 0 1 1 1 3 3 4 4 4 5 5 5 6 6 6 6 7 7 7 8	9 9 1 3 4 5 5 5 6 6 7	7 8 9 9 9 0 0 0 0	2
			4 9 1 2 6 4 0 2 1 5 6 2 9 2 3 4 5 6 7 9 0 1 4 6 7 0 1 2 3 0	4 5 7 9 3 9 3 4 6 1 8 3 5 7 2 6 8 2 3 6 7 1 7 8 0	4 9 1 9 5 0 3 7 8 0	5 2 5 7 9 0 1 6 8	6
			S Y A V G A D Q A M E I Y R E T Q I V T N T D E S N L R G A	T L R Y V L Y D Y R T T R W V L A G T E W Y E T Q	I A A R A P E P K L	E I A L V I G A M	S
B7	B*	702	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - - -	- - S - - - H - - - - - - - E R - - E - - - D K E	- - - - - - - - - -	- V - - - - - -	C
	B*	703	- - S - - S - - - E - - - - N - - - Y - - - - - - - - - -	- - S - - - H - - - - - - - E R - - E - - - D K E	- - - - - - - - - -	- V - - - - - -	C
	B*	704	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - - -	- - S - - - H - - - - - - - E D - - E - - - D K E	- - - - - - - - - -	- V - - - - - -	C
	B*	705	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - - -	- - S - - - H N - - - - - - E R - - E - - - D K E	- - - - - - - - - -	- - - - - - - -	C
	B*	706	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - - -	- - S - - - H N - - - - - - E R - - E - - - D K E	- - - - - - - - - -	- V - - - - - -	C
	B*	707	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - - -	- - - - - - H - - - - - - - E R - - E - - - D K E			
B8	B*	801	- D - M - S - - - E - - - - N - - - F - - - - - - - - - -	- - S - - - H N - - - - L - - D - - - - - - D - E	- - - - - - - - - -	- V - - - - - -	C
	B*	802	- D - M - S - - - E - - - - N - - - F - - - - - N T A L R -	- - S - - - H N - - - - L - - D - - - - - - D - E	- - - - - - - - - -	- V - - - - - -	C
	B*	803	. D - M - S - - - E - - - - N - - - F - - - Y - N I A L R -	- - S - - - H N - - - - - - - D - - - - - - D - E			
B13	B*	1301	- - - M - T - - T - A - - - - - - - S - - - Y - N T A L R -	I I - - L - H N L S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	1302	- - - M - T - - T - A - - - - - - - S - - - Y - N T A L R -	- W T - L - H N L S - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	1303	- - - M - T - - T - A - - - - - - - S - - - Y - N T A L R -	- W T - L - H N L S - - - - - - - L - - - - - -	- - - - - - - - - -	- V - - - - - -	C
B14	B*	1401	- - S - - S - - - E - - - - N - - - C - - - - - - - - - -	- - W - - - - N F S - - - - E - - - - - - H - - -	- - - - - - - - - -	- V - - - - - -	-
	B*	1402	- - - - - S - - - E - - - - N - - - C - - - - - - - - - -	- - W - - - - N F S - - - - E - - - - - - H - - -	- - - - - - - - - -	- V - - - - - -	-
B15	B*	1501	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1502	- - - M - - - - - - A - - - N - - - S - - - Y - - - - - - -	I I - - - - - - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1503	- - - M - S - - - E - - - - - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1504	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- W T - - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1505	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1506	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - F - - - - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1507	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - S - - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1508	- - - M - - - - - - A - - - N - - - F - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1509	- - - M - S - - - E - - - - N - - - C - - - Y - - - - - - -	- - - - - - H N - S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1510	- - - M - S - - - E - - - - N - - - C - - - Y - - - - - - -	- - - - - - H - - S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1511	- - - M - - - - - - A - - - N - - - Y - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1512	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L D G - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1513	- - - M - - - - - - A - - - N - - - S - - - Y - N I A L R -	I I - - - - - - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1514	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L - S - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1515	- - - M - - - - - - A - - - N - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1516	F - - M - - - - - - A - - - - - R N M A S A Y - N I A L R -	- W - - L - H - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1517	- - - M - - - - - - A - - - - - R N M A S A Y - N I A L R -	- - - - - - - H D S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1518	- - - M - S - - - E - - - - N - - - C - - - Y - - - - - - -	- - - - - - H - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1519	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L D G - - -	- - - - - L - - - -	- - - - - - - T -	-
	B*	1520	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	I I - - L - H - S S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	1521	- - - M - - - - - - A - - - N - - - C - - - Y - - - - - - -	I I - - - - - - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1522	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L - - - - - -			
	B*	1523	- - - M - S - - - E - - - - N - - - C - - - Y - N I A L R -	- - - - - - H - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1524	- - - M - - - - - - A - - - - - - - S - - - Y - N I A L R -	- - - - - - H - S S - - - - E W - - L - - - - - -			
	B*	1525	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	I I - - - - - - S S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1526N	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - * - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1527	- - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - F - - H - S S - - - - E W - - L - - - - - -			
	B*	1528	- - - M - - - - - - A - - - - I - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - E W - - L - - - - - -	- - - - - - - - - -		
	B*	1529	- - - M - S - - - E - - - - N - - - F - - - Y - - - - - - -	- - - - - - H - S S - - - - E - - - L - - - - - -	- - - - - - - - - -		
	B*	1530	- - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - - - H N - S - - - - E W - - L - - - - - -			
	B*	1531	- - M - - - - - - A - - - N - - - S - - - Y - - - - - - -	- - - - - - - - - S S - - - - -			
	B*	1532	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - S - - H - S S - - - - E W - - L - - - - - -			
	B*	1533	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - - - H - S - K - - - E W - - L - - - - - -			
	B*	1534	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - - - L - H - S S - - - - E W - - L - - - - - -			
	B*	1535	- - - M - - - - - - A - - - - - - - S - - - Y - - - - - - -	- - T - - - H - S S - - - - E W - - L - - - - - -			
	B*	1537	- - - M - S - - - E - - - - N - - - C - - - Y - - - - - - -	- - - - - - H - - S - - - - E - - - L - - H - - -			
B18	B*	1801	- H S - - S G - - T - - - - N - - - S - - - Y - - - - - - -	- - - - - - H - S S - - - - - - - - - - H - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1802	- H S - - S G - - T - - - - N - - - S - - - Y - - - - - - -	- - N - - - H - S S - - - - - - - - - - H - - -	- - - - - - - - - -	- - - - - - - T -	-
	B*	1803	- H S - - S G - - T - - - - N - - - S - - - - - - - - - - -	- - - - - - H - S S - - - - - - - - - - H - - -			
B27	B*	2701	- H S - - T - L - E - - - - - - - - C A K A Y - N T A L R -	- - N - - - - H D S - - - - - - - - E - - - - - -			
	B*	2702	- H S - - T - L - E - - - - - - - - C A K A - - N I A L R -	- - N - - - - H D S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	2703	- H S - - T - L - E - - H - - - - - C A K A - - D T - L R -	- - N - - - - H D S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	2704	- H S - - T - L - E - - - - - - - - C A K A - - - T - L R -	- - N - - - - H D S - - - - E - - - E - - - - - -	- - G - - - - - - -	- V - - - - - -	C
	B*	27052	- H S - - T - L - E - - - - - - - - C A K A - - D T - L R -	- - N - - - - H D S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	27053	- - - - - L - E - - - - - - - - C A K A - - D T - L R -	- - N - - - - H D S - - - - - - - - E - -			
	B*	2706	- H S - - T - L - E - - - - - - - - C A K A - - - T - L R -	- - N - - - - - - - S - - - - E - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	2707	- H S - - T - L - E - - - - - - - - C A K A - - D T - L R -	- - S - - - H N - - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	2708	- H S - - T - L - E - - - - - - - - C A K A - - - - - - - -	- - N - - - - H D S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	2709	- H S - - T - L - E - - - - - - - - C A K A - - D T - L R -	- - N - - - - H H S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - -	C
	B*	2710	- H S - - T - L - E - - - - - - - - C A K A - - D T - L R -	- - N - - - - H D S - - - - E - - - E - - - - - -			
B35	B*	3501	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H - S S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3502	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L F H N - S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3503	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H - F S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3504	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H N - S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3505	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	- - S - L - H - S S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3506	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H N F S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3507	- - - M V - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H - S S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3508	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H - S S - - - - - R - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	35091	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H N - - - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	35092	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H N - - - - - - - - - L - - - - - -			
	B*	3510	- - - M - - - - - T - - - - - - F - - - Y - - - - - - -	I I - - L - H - S S - - - - - - - L - - -			
	B*	3511	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H - S S - - - - E - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3512	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - - - H N - S - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3513	- - - M - - - - - T - - - - - - - - F - - - Y - - - - - - -	I I - - L - H - F S - - - - - - - L - - - - - -			
	B*	3515	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H - S S - - - - - - - E - - - - - -	V - - - - - - - - -	- - - - - - - T -	-
	B*	3516	- - - M - - - - - T - - - - - - - - F - - - Y - - - - - - -	I I S - - - H - S S - - - - - - - L - - - - - -			
	B*	3517	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I S - - - H - S S - - - - - - - L - - - - - -			
	B*	3518	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	I I - - L - H N - - - - - - - R - - L - - - - - -			
	B*	3519	- - - M - - - - - K - - - - N - - - F - - - Y - - - - - - -	I I - - L - H - S S - - - - - - - L - - - - - -			

			1 1 1 2 3 3 4 4 4 5 5 6 6 6 6 6 6 7 7 7 7 8 8 8 8 9	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 2 2 2 2 2 2 2 2	2 2 2 2 3 3 3 3 3
			4 9 1 2 6 4 0 2 1 5 6 2 9 2 3 4 5 6 7 9 0 1 4 6 7 0 1 2 3 0	9 9 9 9 0 0 1 1 1 3 3 4 4 4 5 5 5 6 6 6 6 7 7 7 8	9 9 1 3 4 5 5 6 6 7	7 8 9 9 9 0 0 0 0 2
			S Y A V G A D Q A M E I Y R E T Q I V T N T D E S N L R G A	T L R Y V L Y D Y R T T R W V L A G T E W Y E T Q	I A A R A P E P K L	E I A L V I G A M S
B37	B*	3701	- H S - - S - - - T - - - - - - - - S - - - Y - D T - L R -	- I - S - - - N F S - - - - - D - - - - - - - - -	- - - - - - - - - -	- - - - - - - T - -
	B*	3702	- H S - - S - - - T - - - - - - - - S - - - Y - D T - L R -	- - N - - - - H D S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - - C
B16	B*	3801	- - S - - S - - - E - - - - N - - - C - - - Y - N I A L R -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	3802	- - S - - S - - - E - - - - N - - - C - - - Y - N T A L R -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	39011	- - S - - S - - - E - - - - N - - - C - - - - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	39013	- - S - - S - - - E - - - - N - - - C - - - - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	39021	- - S - - S - - - E - - - - - - - - S - - - - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	39022	- - S - - S - - - E - - - - - - - - S - - - - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	3903	- - S - - S - - - E - - - - N - - - C - - - - - - - - - -	- - S - - - H N F S - - - - - T - - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	3904	- - - M - S - - - E - - - - N - - - C - - - - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	3905	- - S - - S - - - E - - - - N - - - C - - - Y - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	39061	- - S - - S - - - E - - - - N - - - C - - - - - - - - - -	- W T - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	39062	- - S - - S - - - E - - - - N - - - C - - - - - - - - - -	- W T - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	3907	- - - - - - - E - - - - N - - - C - - - Y - - - - - - -	- - - - - - H - S S - - - - - T - - - - -		
	B*	3908	- - S - - S - - - E - - - - - - - - S - - - Y - - - - - - -	- - - - - - H N F S - - - - - R T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	3909	- - S - - S - - - E - - - - N - - - C - - - - - - - - - -	- - - S - - H N F S - - - - - T - - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	3910	- - S - - S - - - E - - - - N - - - Y - - - - - - - - - -	- - - - - - H N F S - - - - - T - - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
B40	B*	40011	- H - M - T - L T K - - - - - - - - S - - - Y - - - - - - -	- - - - - - H N - - - S - L - - - - E - - - D K E	- - - - - - - - - -	- V - - - - - - C
	B*	40012	- H - M - T - L T K - - - - - - - - S - - - Y - - - - - - -	- - - - - - H N - - - S - L - - - - E - - - D K E	- - - - - - - - - -	- V - - - - - - C
	B*	4002	- H S - - T - L T K - - - - - - - - S - - - Y - - - - - - -	- - S - - - H N - - - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	4003	- H S - - T - L T K - - - - - - - - S - - - Y - - - - - - -	- - S - - - H - S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	4004	- H S - - T - L T K - - - - - - - - S - - - Y - - - - - - -	I I - - L - H N - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	4005	- H S - - T - L T K - - - - - - - - S - - - Y - - - - - - -	- - S - - - H N - - - - - - E - - L - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	4006	- H S - - T - L T K - - - - - - - - S - - - Y - - - - - - -	- W T - - - H N - - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	4007	- H - M - T - L T K - - - - - - - - F - - - Y - - - - - - -	- - - - - - H N - - - S - L - - - - E - - - D K E	- - - - - - - - - -	- V - - - - - - C
	B*	4008	- H S - - T - L T K - - - - N - - - F - - - Y - - - - - - -	- - S - - - H N - - - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	4009	- H S - - T - L T K - - - - - - - - S - - - Y - - - - - - -	- - S - - - - - - - - - - - - - E - - - - - -		
B41	B*	4101	- H - M - T - L T K - - - - - - - - S - - Y - - - - - - -	- W - - - H N - - - - - - - D - - - - - - D - E	- - - - - - - - - -	- V - - - - - - C
	B*	4102	- H - M - T - L T K - - - - - - - - S - - - Y - - - - - - -	- - S - - - H N - - - - - - D - - - - - - D - E	- - - - - - - - - -	- V - - - - - - C
B42	B*	4201	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - -	- - S - - - H N - - - - - - - D - - - - - - D - E	- - - - - - - - - -	- V - - - - - - C
B12	B*	4402	- - - M - T - L T K - - - - - - - - S - - - Y - N T A L R -	I I - - - - - - D S - - - - - D - - L - S - - - -	- V - - - - - - - -	- V - - - - - - C
	B*	44031	- - - M - T - L T K - - - - - - - - S - - - Y - N T A L R -	I I - - - - - - D S - - - - - - - - L - S - - - -	- V - - - - - - - -	- V - - - - - - C
	B*	44032	- - - M - T - L T K - - - - - - - - S - - - Y - N T A L R -	I I - - - - - - D S - - - - - - - - L - S - - - -	- V - - - - - - - -	- V - - - - - - C
	B*	4404	- - - M - T - L T K - - - - - - - - S - - - Y - N T A L R	I I - - - - - - D S - - - - - R - - - - S - - - -	- V - - - - - - - -	- V - - - - - - C
	B*	4405	- - - - - - - - - - - - - - - - S - - - Y - N T A L R -	I I - - - - - - - S - - - - - D - - L - S - - - -		
	B*	4406	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	I I - - - - - - D S - - - - - D - - L - S - - - -		
	B*	4407	- - - M - T - L - K - - - - - - - - S - - - Y - N T A L R -	I I - - - - - - D S - - - - - - - - L - S - - - -	- V - - - - - - - -	- V - - - - - - C
	B*	4408	- - - M - T - L - - A - - - - - - - S - - - Y - N T A L R -	I I - - - - - - D S - - - - - D - - L - S - - - -	- V - - - - - - - -	- V - - - - - - C
	B*	4410	- - - M - T - L T K - - - - - - - - S - - - Y - N T A L R -	I I - F L - H N L S - - - - - - - - L - S - - - -		
	B*	4501	- H - M - T - L T K - - - - - - - - S - - - Y - - - - - - -	- W - - L - - N L S - - - - - D - - L - S - - - -	- - - - - - - - - -	- - - - - - - T - -
B46	B*	4601	- - - M - - - - - - A - - - - - - K Y R Q A - V - - - - - -	- - - - - - H - S S - - - - E W - - L - - - - -	- - - - - - - - - -	- - - - - - - T - -
B47	B*	4701	- - - M - T - L T K - - - - - - - - S - - - Y - D T - L R -	- - - F - - - H D S - - - - - - - - E - - - - - -	- - - - - - - - - -	- V - - - - - - V C
B48	B*	4801	- - S - - S - - - E - - - - - - - - S - - - Y - - - - - - -	- - S - - - H N - - - S - L - - - - E - - - D K E	- - - - T - - - - -	- V - - - - - - C
	B*	4802	- - S - - S - - - E - - - - - - - - S - - - Y - - - - - - -	I I - - L - H - S S - - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	4803	- - S - - S - - - E - - - - - - - - S - - - Y - - - - - - -	- - - - - - H N - - - S - L - - - - E - - - D K E		
B21	B*	4901	- H - M - T - L T K - - - - - - - - S - - - Y - N I A L R -	- W - - L - - N L S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T - -
	B*	5001	- H - M - T - L T K - - - - - - - - S - - - Y - - - - - - -	- W - - L - - N L S - - - - E - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T - -
B5	B*	51011	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - - H - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	51012	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - - H - - -		
	B*	51021	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	51022	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	5103	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - G H - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	5104	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	I I - - - - H N - S - - - - E - - - L - - H - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	5105	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- W T - - - H N - S - - - - R - - L - - - - - -	V - - - - - - - - -	
	B*	5106	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- - - - - - H N - S - - - - E - - - L - - H - - -		
	B*	5107	- - - M - - - - - T - - - - N - - - S - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - - H - - -		
	B*	5108	. . . M - - - - - T - - - - N - - - F - - - Y - N I A L R -	- W T - - - H N - S - - - - D - - L - - H - - -		
	B*	52011	- - - M - - - - - T - - - - - - - - S - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - - H - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	52012	- - - M - - - - - T - - - - - - - - S - - - Y - N I A L R -	- W T - - - H N - S - - - - E - - - L - - H - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	5301	- - - M - - - - - T - - - - N - - - F - - - Y - N I A L R -	I I - - L - H - S S - - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T - -
B22	B*	5401	- - - M - - - - - G - V - - N - - - Y A Q A - - - - - - -	- W T - L - H N L S - - - - - - - - - - - - - - -	- - - - - - - - - -	- - - - - - - T - -
	B*	5501	- - - M - - - - - E - - - - N - - - Y A Q A - - - - - - -	- W T - L - H N L S - - - - E - - - - - - - - - -	- - - - - - - - - -	- - - - - - - T - -
	B*	5502	- - - M - - - - - E - - - - N - - - Y A Q A - - - - - - -	- W T - L - H N L S - - - - - - - - - - - - - - -	- - - - - - - - - -	- - - - - - - T - -
	B*	5503	- - - M - - - - - E - - - - N - - - Y A Q A - V - - - - - -	- W T - L - H N L S - - - - E - - - - - - - - - -		
	B*	5504	- - - M - - - - - E - - - - N - - - Y A Q A - - - - - - - -	- - S - - - H N - - - - - - - - - - - - - - - - -		
	B*	5601	- - - M - - - - - E - - - - N - - - Y A Q A - - - - - - - -	- W T - L - H N L S - - - - - - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T - -
	B*	5602	- - - M - - - - - E - - - - N - - - Y A Q A - - - - - - - -	- - - - L - H N L S - - - - - - - - L - - - - - -	- - - - - - - - - -	- - - - - - - T - -
B17	B*	5701	- - - M - - - - - - A - - G - - R N M A S A Y - N I A L R -	I I V - - - H - S S - - - - - - - - L - - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	5702	- - - M - - - - - - A - - G - - R N M A S A Y - N I A L R -	I I V - - - H N - S - - - - - R - - L - - - - - -	- - - - - - - - - -	- V - - - - - - C
	B*	5703	- - - M - - - - - - A - - G - - R N M A S A Y - N I A L R -	I I V - - - H N - S - - - - - - - - L - - - - - -		
	B*	5704	- - - M - - - - - - A - - G - - R N M A S A Y - N I A L R -	I I V - - - - - - D S - - - - - R - - L - - - - -		
	B*	5801	- - - M - - - - - T - - - G - - R N M A S A Y - N I A L R -	I I - - L - H - S S - - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	5802	- - - M - - - - - T - - - G - - R N M A S A Y - N I A L R -	- - W - L - H - S S - - - - - - - - L - - - - - -	V - - - - - - - - -	- - - - - - - T - -
B59	B*	5901	- - - M - - - - - E - - - - N - - - F - - - Y - N I A L R -	- W T - L - H N L S - - - - - - - - - - - - - - -	- - - - - - - - - -	- - - - - - - T - -
B67	B*	67011	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -	- - - - - - - - - -	- V - - - - - - -
	B*	67012	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - -	- - - - - - H N F S - - - - - - T - - - - - - - -		
B73	B*	7301	- H S - - T - - - E - - - - N - - - C A K A - V G - - - - D	- W T - M - - N F - - - - - - - - - E - - H - - -	- - - G - - Q Q E C	K - V T A V V - - -
B78	B*	7801	- - - M - - - - - T - - - - N - - - F - - - - - - - - - -	- W T - - - H N - S - - - - E - - - L - - H - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	78021	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	- W T - - - H N - S - - - - E - - - L - - H - - -	V - - - - - - - - -	- - - - - - - T - -
	B*	78022	- - - M - - - - - T - - - - N - - - F - - - Y - - - - - - -	- W T - - - H N - S - - - - E - - - L - - H - - -		
B81	B*	8101	- - S - - S - - - E - - - - N - - - Y A Q A - - - - - - -	- - S - - - H N - - - S - L - - - - E - - - D K E	- - - - T - - - - -	- V - - - - - - C
B82	B*	8201	- - - M - S - - - E - - - - N - - - Y A Q A - - - - - - -	- - - F L - H N L S - - - - - D - D L - S - - - -		

TABLE 8 POLYMORPHIC RESIDUES AT HLA-C

		alpha 1	alpha 2	alpha 3	TM	C
			1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 2 2 2 2 2 2 2 2	2 2 2 3 3 3 3 3	3 3 3 3
		1 1 1 1 2 2 3 4 5 6 6 7 7 8 9	9 9 9 9 9 0 1 1 1 3 4 4 5 5 5 6 7 7 7 7	8 9 9 1 1 4 5 6 6 7 7	7 8 9 0 0 0 0 0	2 2 3 4
		1 6 9 1 4 6 7 1 4 5 9 0 6 8 3 7 0 0	1 4 5 7 9 3 3 4 6 8 3 7 2 5 6 3 0 3 5 7	4 3 4 1 9 8 3 1 7 0 3	5 5 5 3 4 5 6 7	4 6 9 0
		G R Y A R G R R A R A P I K T S N A	G T L R Y V Y D Y T T W V Q L T R E G E	P P I A R V E V P L R	E V A V V A A V	A S A C
Cw1	Cw*0102	C K F S - - - - S - - - K - - - - -	- - - W C L - - - - - - E - R - - - - -	H - V - W M - - - - -	- - - - - - V -	- - - -
	Cw*0103	C K F S - - - - S - - - K - - - - -	- - - W C L - N F - - - E - R - - - - -	H - V - W M - - - - -	- - - - - - V -	- - - -
Cw2	Cw*0202	C - - - - S - H - - - - K - - N K -	- - - - - L - - S - - - E - W E - - - -	H - V T - - - - - - -	- - - - - - V -	- - - -
	Cw*0203	- - - - S - H - - - - K - - N K -	- - - - - L - - S - - - E - W E - - - -			
Cw3	Cw*0302	- - - - - - - H - - - - K - - - - -	- I - - - - - - S - - - E - - L - K - -	H - V - W - - - - - -	- - - - - - V -	- - - -
	Cw*0303	- - - - - - - H - - - - K - - - - -	R I I - - - - - - - - - E - - L - K - -	H - V - W - - - - - -	- - - - - - V -	- - - -
	Cw*0304	- - - - - - - H - - - - K - - - - -	- I I - - - - - - - - - E - - L - K - -	H - V - W - - - - - -	- - - - - - V -	- - - -
Cw4	Cw*0401	- - S S W - - - - - E - K - A N K D	- - - - F L - N F - - - E - R - - - - -	H - V - W - - - - - -	K - - M - - V -	- - - -
	Cw*0402	- - S S W - - - - - S R K N A N K D	- - - - F L - N F - - - E E R - - - - -	H - V - W - - - - - -	K - - M - - V -	- - - S
	Cw*0403	- - - - - S - H - - - - K - A N K D	- - - - F L - N F - - - E - R - - - - -	H - V - W - - - - - -	K - - - - - V -	- - - -
Cw5	Cw*0501	C - - - - - - - - Q - - K - - N K -	- - - - - L - N F K - - E - R - - - - K	H - V - - - - - - - -	G - - - - - V -	- - - -
Cw6	Cw*0602	C - D - - - - - S - - - K - A N K D	- - - W - L - - S - - - E - W - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
Cw7	Cw*0701	C - D - - - - - S - - - N - A - - D	- - - - - L - - S - - L A - - - - - - -	- - L - - - Q M Q - S	- - V - - T - M	- C T -
	Cw*0702	C - D - - - - - S - - - K - A - - D	- - - - S L - - S - - L A - - - - - - -	- - L - - - Q M Q - S	- M V - - T - M	- C T -
	Cw*0703	C - D - - - A - S - - - K - A - - D	- - - - S L - - S - - - A - - L - - - -	- - L - - - Q M Q - S	- M V - - T - M	- C T -
	Cw*0704	C - D - - - - - S - - - K - A - - D	- - F - - L - - F - - L A - D - - - - K	- - L - - - Q M Q - S	- M V - - T - M	- C T -
	Cw*0705	- D - - - - - S - - - K - A - - D	- - - N - L - - S - - L A - - - - - - -			
	Cw*0706	C - D - - - - - S - - - N - A - - D	- - - - - L - - S - - L A - - - - - - -	- - L - - - Q M Q - S	- M V - - T - K	V C T -
Cw8	Cw*0801	C - - - - - - - - Q - - K - - - - -	- - - - - L - N F - - - T - - - - - - K	H - V - - - - - - - -	G - - - M - V -	- - - -
	Cw*0802	C - - - - - - - - Q - - K - - - - -	- - - - - L - N F K - - E - R - - - - K	H - V - - - - - - - -	G - - - M - V -	- - - -
	Cw*0803	C - - - - - - - - Q - - K - - - - -	- - - - - L - N F - - - T - - - - - R K	H - V - - - - - - - -	G - - - M - V -	- - - -
Cw12	Cw*12021	C - - - - - - - - - - - K - A - - -	- - - - - L - - S - - - E - W - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
	Cw*12022	C - - - - - - - - - - - K - A - - -	- - - - - L - - S - - - E - W - - - - -	H - V - - - - - - - -	- - - M - V -	- - - -
	Cw*1203	C - - - - - - - - - - - K - A - - -	- - - W - L - - S - - - E - W - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
Cw13	Cw*1301	C - - - - - - H - - - - K - A - - -	- - - - - L - - S - - - E - W - - - - -	H - V - - - - - - - -	- - - - - - V -	- - - -
Cw14	Cw*1402	C - S S - - - - - - - - K - - - - -	- - - W F L - - S - - - E - R - - - - -	H - V - W - - - - - -	- - - - - - V -	- - - -
	Cw*1403	C - S S - - - H - - - - K - - - - -	- - - W F L - - S - - - E - R - - - - -	H - V - W - - - - - -	- - - - - - V -	- - - -
Cw15	Cw*1502	C - - - - - - H - - - - N - - N K -	- I I - - L H - L - - - E - - - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
	Cw*1503	C - - - - - - H - - - - N - A N K -	- I I - - L H - L - - - E - - - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
	Cw*1504	C - - - - - - H - - - - N - - N K -	- I I - - L - - S - - - E - - - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
	Cw*15051	C - - - - - - H - - - - N - - N K -	- I I - - L H - F - - - E - - - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
	Cw*15052	C - - - - - - H - - - - N - - N K -	- I I - - L H - F - - - E - - - - - - -	H - V - - - - - - - -	- - - - M - V -	- - - -
Cw16	Cw*1601	C - - - - - - - - - - - K - - - - -	- - - W - L - - S - - - A - Q - - - - -	H L V - - - - - - - -	- - - - - - V -	- - - -
	Cw*1602	C - - - - - - - - - - - K - - N K -	- - - W - L - - S - - - A - Q - - - - -	H L V - - - - - - - -	- - - - - - V -	- - - -
Cw17	Cw*1701	- - - - - - - - - - - - K - A N K -	- - I - - L - N F - S L E - - E G - - -	R - V - - - Q - Q C -		
	Cw*1702	- - - - - - - - - - - - K - A N K -	- - I - - L - N F - S L E - - E G - - -	R - V - - - Q - Q C -		
Cw18	Cw*1801	C - D - - - - - S - - - K - A N K D	- - - - F L - N F - - - E - R - - - - -	H - V - W - - - - - -	K - V - - - V -	- - - -

Table 9
Cumulative frequencies of HLA-A alleles in Caucasian, Black, and Asian populations

Caucasian

French			Spanish			Brazilian			USA			Canadian		
Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP
A2	0.213	0.38	A2	0.276	0.48	A2	0.260	0.45	A2	0.283	0.49	A2	0.267	0.46
A1	0.137	0.58	A1	0.113	0.63	A1	0.099	0.59	A1	0.169	0.70	A1	0.186	0.70
A3	0.133	0.73	A29	0.093	0.73	A24	0.096	0.70	A3	0.122	0.82	A3	0.113	0.81
A24	0.089	0.82	A3	0.090	0.82	A3	0.069	0.77	A24	0.096	0.89	A24	0.113	0.90
A29	0.064	0.87	A11	0.070	0.87	A28	0.064	0.83	A11	0.055	0.92	A32	0.058	0.93
A11	0.062	0.91	A24	0.068	0.92	A30	0.060	0.88	A32	0.051	0.95	A26	0.053	0.96
A28	0.056	0.94	A30	0.051	0.94	A26	0.053	0.91						
A26	0.044	0.96	A26	0.045	0.96	A31	0.048	0.94						
						A11	0.047	0.96						

Black

South African			San Bushmen			Capetown			Brazilian			USA		
Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP
A2	0.149	0.28	A30	0.204	0.37	A2	0.165	0.30	A23	0.144	0.27	A2	0.167	0.31
A30	0.136	0.49	A2	0.184	0.63	A30	0.113	0.48	A2	0.128	0.47	A28	0.109	0.48
A28	0.111	0.64	A3	0.155	0.79	A1	0.099	0.61	A30	0.109	0.62	A30	0.095	0.60
A23	0.097	0.74	A23	0.126	0.89	A28	0.092	0.72	A28	0.090	0.72	A3	0.089	0.71
A34	0.069	0.81	A43	0.117	0.95	A26	0.070	0.79	A3	0.080	0.80	A23	0.081	0.79
A3	0.062	0.86				A24	0.062	0.84	A33	0.060	0.85	A33	0.081	0.86
A29	0.059	0.90				A33	0.060	0.89	A1	0.056	0.89	A1	0.053	0.89
A29	0.050	0.93				A3	0.057	0.92	A31	0.054	0.92	A34	0.051	0.92
A24	0.041	0.95				A11	0.053	0.95	A24	0.035	0.94	A24	0.047	0.95
									A11	0.034	0.96			

Asian

Japanese			Korean			Northern Han (Chinese)			Southern Han (Chinese)			Thai		
Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP	Allele	Freq	CP
A24	0.351	0.58	A2	0.293	0.50	A2	0.367	0.60	A2	0.337	0.56	A11	0.325	0.54
A2	0.244	0.84	A24	0.228	0.77	A11	0.203	0.82	A11	0.319	0.88	A2	0.255	0.82
A26	0.109	0.91	A33	0.149	0.89	A24	0.126	0.91	A24	0.199	0.98	A24	0.146	0.92
A11	0.104	0.96	A11	0.094	0.94	A1	0.047	0.93				A33	0.136	0.98
			A26	0.081	0.98	A30	0.044	0.10						

Table 10
Cumulative frequencies of HLA-B alleles in Caucasian, Black, and Asian populations

Caucasian

French

Allele	Freq	CP
B44	0.108	0.20
B35	0.084	0.35
B7	0.075	0.46
B14	0.072	0.56
B51	0.069	0.65
B8	0.068	0.73
B62	0.053	0.78
B18	0.048	0.82
B55	0.037	0.85
B27	0.036	0.88
B13	0.030	0.90
B38	0.030	0.92
B49	0.030	0.93
B60	0.030	0.95

Spanish

Allele	Freq	CP
B44	0.170	0.31
B35	0.084	0.44
B51	0.078	0.55
B7	0.076	0.65
B14	0.069	0.73
B8	0.064	0.79
B18	0.055	0.84
B49	0.037	0.87
B62	0.037	0.89
B38	0.030	0.91
B27	0.028	0.93
B50	0.027	0.94
B13	0.025	0.95

Brazilian

Allele	Freq	CP
B35	0.137	0.26
B44	0.106	0.43
B51	0.071	0.53
B18	0.053	0.60
B7	0.051	0.66
B14	0.050	0.72
B8	0.049	0.77
B39	0.049	0.81
B62	0.042	0.85
B38	0.036	0.87
B27	0.031	0.89
B49	0.030	0.91
B60	0.024	0.93
B57	0.022	0.94
B45	0.021	0.95

USA

Allele	Freq	CP
B44	0.104	0.20
B7	0.100	0.37
B8	0.100	0.52
B35	0.085	0.63
B62	0.055	0.69
B18	0.049	0.74
B60	0.045	0.79
B14	0.041	0.82
B27	0.041	0.86
B38	0.041	0.89
B57	0.039	0.91
B51	0.037	0.93
B61	0.033	0.95
B13	0.030	0.96

Canadian

Allele	Freq	CP
B44	0.133	0.25
B7	0.111	0.43
B8	0.111	0.58
B35	0.076	0.68
B62	0.070	0.75
B14	0.065	0.81
B18	0.058	0.86
B60	0.056	0.90
B27	0.043	0.92
B51	0.040	0.94
B57	0.036	0.96

Black

South African

Allele	Freq	CP
B70	0.226	0.40
B58	0.172	0.64
B42	0.128	0.78
B8	0.098	0.86
B7	0.081	0.91
B44	0.071	0.95

San Bushmen

Allele	Freq	CP
B58	0.356	0.59
B8	0.133	0.74
B7	0.128	0.85
B70	0.104	0.92
B18	0.071	0.96

Capetown

Allele	Freq	CP
B58	0.110	0.21
B44	0.086	0.35
B70	0.086	0.48
B51	0.062	0.57
B8	0.057	0.64
B18	0.057	0.71
B35	0.048	0.76
B57	0.048	0.80
B7	0.043	0.84
B14	0.043	0.87
B42	0.038	0.90
B27	0.029	0.91
B60	0.029	0.93
B13	0.024	0.94
B37	0.024	0.95

Brazilian

Allele	Freq	CP
B35	0.100	0.19
B7	0.096	0.35
B44	0.070	0.46
B42	0.060	0.55
B53	0.044	0.61
B51*	0.041	0.66
B52	0.036	0.70
B18	0.034	0.74
B49	0.031	0.77
B55	0.031	0.80
B57	0.031	0.82
B14	0.026	0.85
B45	0.026	0.86
B8	0.022	0.88
B70	0.022	0.90
B13	0.018	0.91
B50	0.017	0.92
B51	0.017	0.93
B58	0.017	0.93
B62	0.017	0.94
B39	0.013	0.95

USA

Allele	Freq	CP
B53	0.128	0.24
B7	0.083	0.38
B70	0.082	0.50
B35	0.077	0.60
B58	0.069	0.69
B44	0.062	0.75
B42	0.047	0.80
B57	0.042	0.83
B45	0.038	0.86
B18	0.033	0.89
B8	0.032	0.91
B51	0.032	0.92
B14	0.030	0.94
B63	0.028	0.95

Asian

Japanese

Allele	Freq	CP
B52	0.107	0.20
B61	0.107	0.38
B51	0.093	0.52
B62	0.083	0.63
B35	0.081	0.72
B44	0.074	0.79
B54	0.063	0.85
B60	0.056	0.89
B7	0.050	0.92
B39	0.045	0.94
B46	0.044	0.96

Korean

Allele	Freq	CP
B62	0.105	0.20
B44	0.099	0.37
B61	0.092	0.50
B51	0.078	0.61
B35	0.070	0.69
B54	0.065	0.76
B13	0.063	0.82
B58	0.052	0.86
B60	0.042	0.89
B7	0.041	0.91
B46	0.040	0.94
B48	0.040	0.95

Northern Han (Chinese)

Allele	Freq	CP
B13	0.164	0.30
B51	0.118	0.48
B62	0.118	0.64
B60	0.060	0.71
B61	0.042	0.75
B44	0.041	0.79
B35	0.036	0.82
B55	0.032	0.85
B58	0.032	0.87
B7	0.031	0.89
B46	0.028	0.91
B48	0.028	0.93
B57	0.028	0.94
B63	0.026	0.95

Southern Han (Chinese)

Allele	Freq	CP
B60	0.171	0.31
B46	0.154	0.54
B62	0.147	0.72
B13	0.085	0.80
B61	0.054	0.85
B54	0.049	0.88
B58	0.049	0.92
B51	0.043	0.94
B55	0.040	0.96

Thai

Allele	Freq	CP
B46	0.140	0.26
B13	0.093	0.41
B60	0.083	0.53
B75	0.083	0.64
B51	0.064	0.71
B27	0.060	0.77
B44	0.054	0.82
B57	0.052	0.86
B62	0.050	0.90
B61	0.043	0.92
B38	0.035	0.94
B52	0.031	0.96

Table 11

Primers and Probes for molecular HLA-A and HLA-B typing

				wash conditions		
Probe	Codons	sequence (5' - 3')	Annealing	temp	[SSPE]	# washes
HLA-A, exon 2	forward primer:	HLA-A Amp A long, codons 37-42				
	reverse primer:	Amp B, intron 2				
441	42-46	AGC CAG AAG ATG GAG	56C 5 min	38	2.1x	1x
561B	54-58	AG GAG GGT CCG GA	56C 5 min	38	0.1x	1x
562	55-59	GAG AGG CCT GAG TAT	56C 5 min	38	2.1x	1x
621	60-65	G GAC CTG CAG ACA C	56C 5 min	38	2.1x	1x
622	59-64	T TGG GAC GAG GAG ACA	56C 5 min	38	0.01x	1x
623	59-63	TAT TGG GAC GGG GAG	56C 5 min	38	0.1x	1x
624	59-64	AT TGG GAC CAG GAG ACA	56C 5 min	38	0.1x	1x
631	62-66	G CGG AAC ACA CGG AA	56C 5 min	38	2.1x	1x
701	68-73	AAG GCC CAG TCA CAG A	56C 5 min	47	0.1x	1x
731	71-76	TCA CAG ATT GAC CGA GT	56C 5 min	38	2.1x	1x
762	75-79	CGA GAG AAC CTG C	56C 5 min	38	1.1x	1x
763	74-78	AC CGA GCG AAC CTG	56C 5 min	38	2.1x	1x
771	75-79	GA GAG AGC CTG C	56C 5 min	38	0.4x	1x
801B	79-84	CGG ATC GCG CTC CGC TA	56C 5 min	38	0.005x	2x
HLA-A, exon 3	forward primer:	variable				
	reverse primer:	Aex3.92or Ain3.11, both in intron 3				
901	3-7	CAC ACC GTC CAG A	56C 5 min		no wash	
902	3-7	CAC ACC CTC CAG A	56C 5 min	38	2.1x	1x
903	3-7	CAC ACC ATC CAG A	56C 5 min			
920	5-10	TC CAG ATG ATG TAT GGC	56C 5 min	38	2.1x	1x
931	7-12	G ATG TTT GGC TGC GA	56C 5 min	38	0.01x	1x
932	6-11	G AGG ATG TGT GGC T	56C 5 min	38	2.1x	1x
941	10-13	GC TGC CAC GTG	56C 5 min		no wash	
971	13-16	TG GGG CCG GA	56C 5 min			
972	13-16	TG GGG TCG GAC	56C 5 min			
981	17-20	AC GGG CGC CTC	56C 5 min	38	0.005x	1x
982	15-19	TCG GAC TGG CG	56C 5 min	38	2.1x	1x
991	19-22	TC CTG CGC GG	56C 5 min		no wash	
1001	23-26	TAC CGG CAG GAC	56C 5 min			
1003	23-27	G TAT GAA CAG CAC GC	56C 5 min	38	0.1x	1x
1004	23-27	TAC CAG CAG GAC G	56C 5 min	38	0.1x	1x
1005	24-28	CAG CAG AAC GCT TAC	56C 5 min	38	2.1x	1x
1100	26-30	GAC GCT TAC GAC G	56C 5 min	38	2.1x	1x
1122	25-29	AG GAC GCC TAC G	56C 5 min			
1123	24-28	AA CAG CAC GCC TAC	56C 5 min	38	0.1x	1x
1200	34-38	ATC GCC TTG AAC GAG	56C 5 min	38	.005x	1x
1222	33-37	TAC ATC GCC CTG AA	56C 5 min			
1300	41-44	CGC TCT TGG ACC	56C 5 min			
1351	47-51	C ATG GCG GCT CA	56C 5 min	38	.01x	1x
1352	46-50	CG GAC AAG GCA G	56C 5 min	38	0.1x	1x
1363	50-53	GCT CAG ACC ACC	56C 5 min			
1380	53-56	C ACC AAG CAC AAG	56C 5 min			
1382	51-56	G ATC ACC AAG CGC AA	56C 5 min	38	2.1x	1x
1383	52-56	C ACC AAG CGC AAG	56C 5 min	38	.005x	1x
1390	57-60	G GAG ACG GCC	56C 5 min	38	0.1x	1x
1400	58-62	G GCG GTC CAT G	56C 5 min			
1421	59-63	G GCC CAT GTG G	56C 5 min	38	2.1x	1x
1430	60-63	C CGT TGG GCG	56C 5 min	38	2.1x	1x
1431	60-63	C CGT CGG GC	56C 5 min	38	0.1x	1x
1432	61-64	CAT GAG GCG GAG	56C 5 min		no wash	

1433	60-63	C CAT GCG GCG	56C 5 min	38	0.1x	1x
1470	64-68	GAG CAG TTG AGA GC	56C 5 min	38	2.1x	1x
1471	64-68	AG CAG TGG AGA GC	56C 5 min	38	0.1x	1x
1472	64-68	GAG CAG CAG AGAG	56C 5 min	38	2.1x	1x
1473	72-75	GGC GAG TGC GT	56C 5 min	38	2.1x	1x
1474	75-78	TG GAC GGG CTC	56C 5 min	38	0.1x	1x
1475	72-75	GGC CTG TGC GT	56C 5 min	38	2.1x	1x

HLA-B, exon 2 forward primer: 259, 260, 261 or 262 (around codons 39-46)
reverse primer: 202 RPL, codons 83-90

480	48-53	G GCG CCA TGG ATA G	56C 5 min	38	2.1x	1x
520	51-56	A ATA GAG CAA GAG GGG	56C 5 min	38	1.1x	1x
570	56-61	GG CCG GAA TAT TGG G	56C 5 min	38	0.01x	2x
710	71-76	CA CAG ACT TAC CGA GA	56C 5 min	38	0.005x	1x
610	61-65	GAC CGG AAC ACA CAG	56C 5 min	38	1.1x	1x
612	64-69	A CAG ATC TCC AAG ACC	56C 5 min	38	0.1x	1x
650	65-69	CGG AAC ATG AAG GCC	56C 5 min	38	2.1x	1x
655	64-70	A CAG ATC TTC AAG ACC C	56C 5 min	38	0.005x	1x
680	68-72	C AAG GCC AAG GCA CA	56C 5 min	57	0.01x	1x
681	68-72	G GCC CAG GCA CAG	56C 5 min	47	0.005x	1x
780	78-82	TG CGG ACC CTG CT	56C 5 min		no wash	
781	80-83	ACC GCT GCC CGC	56C 5 min		no wash	
782	78-82	TG CGC ACC GCG CT	56C 5 min		no wash	
790	79-83	CGG AAC CTG CTG CGC G	56C 5 min	38	0.005x	2x
791	78-82	TG CGG ATC GCG CTC	56C 5 min		no wash	
792	79-83	CGG AAC CTG CGC GG	56C 5 min	38	2.1x	1x

HLA-B, exon 3 forward primer: Bin2.184 , intron 2
reverse primer: Bin3.47, intron 3

3040	1-6	TCT CAC ATC ATC CAG	56C 5 min	38	2.1x	1x
3041	2-6	CT CAC ACT TGG CAG	56C 5 min	38	2.1x	1x
3042	2-6	TCT CAC ACC CTC CAG	56C 5 min	38	2.1x	1x
3070	5-10	G CAG ACG ATG TAT GG	56C 5 min	38	2.1x	1x
3071	6-10	CAG AGC ATG TAC GG	56C 5 min	38	2.1x	1x
3072	5-10	CTC CAG TGG ATG TAT G	56C 5 min		no wash	
3073	5-10	CTC CAG AAT ATG TAT GGC	56C 5 min		no wash	
3071	6-11	G AGG ATG TTT GGC TG	56C 5 min	38	2.1x	1x
3094	8-12	ATG TAC GGC TGC GAC	56C 5 min	38	2.1x	1x
3095	8-12	ATG TAT GGC TGC GAC	56C 5 min	38	2.1x	1x
3131	10-15	C TGC GAC CTG GGG C	56C 5 min	38	0.1x	1x
3230	21-26	GC GGG CAT AAC CAG T	56C 5 min		no wash	
3231	21-25	CGC GGG CAT GAC CA	56C 5 min	38	0.4x	1x
3233	21-25	C GGG TAT GAC CAG	56C 5 min	38	2.1x	1x
3260	24-28	C CAG TAC GCC TAC	56C 5 min	38	2.1x	1x
3261	24-29	AAC CAG TTA GCC TAC G	56C 5 min	38	2.1x	1x
3262	24-28	AC CAG TCC GCC TAC	56C 5 min		no wash	
3310	29-35	C GGC AAA GAT TAC ATC G	56C 5 min	38	2.1x	1x
3400	33-40	C ATC GCC CTG AAC GAG GAC CTG	56C 5 min	38	2.1x	1x
3411	38-42	AG GAC CTG CGC TCC	56C 5 min	38	2.1x	1x
3490	48-52	AC ACC GCC GCT CAG A	56C 5 min		no wash	
3530	50-54	CT CAG ATC TCC CAG	56C 5 min	38	1.1x	1x
3620	60-64	CC CGT GAG GCG GAC	56C 5 min	38	2.1x	1x
3662	64-68	GAG CAG TGG AGA G	56C 5 min	38	2.1x	1x
3664	65-70	G CTG AGA ACC TAC C	56C 5 min	38	2.1x	1x

Table12

Variability in amino acid sequence in HLA-A2 subtypes and ability to bind peptides

	Sequences																				Subtype frequencies in HLA-A2+ individuals					Peptide binding						
	a1					a2														a3												
									1	1	1	1	1	1	1	1	1	2													
	4	6	7	7		9	9	9	0	4	4	4	5	5	6	6	6	3													
allele	9	3	6	3	4	5	7	9	7	2	5	9	2	6	3	6	7	5		Cauc Oxford	Africa Gambia	Africa Nairobi	China Sing.	Japan	influenza matrix 58-66	HIV IIIB p18 I10	HIV AP10 MN	HIV SP50 MN	HPV E6 29-38	HPV E7 12-20	tumor Mage
	F	Q	K	T	H	V	R	Y	W	T	H	A	V	L	T	E	W	A		N=76	N=20	N=85	N=66	N=53						
A*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		.96	.40	.55	.23	.47	1.43	1.25	1.11	1.11	1.43	varies	1.02
A*0202	-	R	-	-	-	L	-	-	-	-	-	-	-	W	-	-	-	-		-	.40	.28	-	-	1.79	1.05	.97	1.03	.94	1.02	.95
A*0203	-	-	-	-	-	-	-	-	-	-	-	T	E	W	-	-	-	-		-	-	-	.23	-	1.10	1.09	1.01	.98	1.04	.96	.99
A*0204	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-		population distribution not known					1.31	.99	1.00	1.03	.84	.79	1.05
A*0205	Y	R	-	-	-	L	-	-	-	-	-	-	-	W	-	-	-	-		.01	.20	.08	-	-	1.40	1.01	1.09	1.06	1.03	1.11	1.00
A*0206	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	}	-	-	-	.08	.36	1.68	.97	.98	.94	.94	.97	1.02
A*0207	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	}	-	-	-	.46	.17							
A*0217	-	-	-	-	-	L	M	F	-	-	-	-	-	-	-	-	-	-		present in Native Americans?					1.32	1.04	1.04	.88	1.03	.80	1.02
A*0208	Y	R	N	-	-	L	-	-	-	-	-	-	-	W	-	-	-	-													
A*0209	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E													
A*0210	Y	-	-	-	-	-	-	F	G	-	-	-	-	-	-	-	-	-													
A*0211	-	-	-	I	D	-	-	-	-	-	-	-	-	-	-	-	-	-													
A*0212	-	-	-	-	-	-	-	-	-	-	-	-	-	Q	-	-	-	-													
A*0213	-	-	-	-	-	-	-	-	-	-	-	-	E	Q	-	-	-	-													
A*0214	Y	R	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-													
A*0215	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-													
A*0216	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	-	-	-													
A*0219	-	-	-	-	-	-	-	-	-	I	D	-	-	Q	-	D	G	-													
A*0220	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													

Ratios >1.19 are considered significant
Ratios between 1.10 and 1.19 may indicate stable interactions
with an HLA-A2 molecule, but the functional significance is not known